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*EDITORS:*
T. DALLING, *M.A., M.R.C.V.S.* J. McCUNN, *M.R.C.V.S., L.R.C.P.*

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## *EDITORIAL*

### REACTION TO ENVIRONMENT

ALL living things, be they plant or animal, react to change of environment. Such reaction may be beneficial, or the reverse. The latter may be so severe that it may lead to ultimate extinction. The former may provide such changes that, after a time, the resulting product may bear little resemblance to its ancestors. Those that have eyes to see can observe these changes in their own garden, and within the scope of a few years. In the case of the animal kingdom, for obvious reasons, the state of change may, and does, necessitate a longer period of time and be not apparent in one man's lifetime. Stock owners, as well as gardeners, are not unconscious of these basic facts, and by judiciously combining the forces of reaction to environment with selective breeding they have been able to alter the characteristics of many species of animals so that modern stock differs very materially, physically and functionally from their ancestors.

All so-called improvements of plants or animals depend upon these basic principles. Now, such changes, even if in the main they appear to be beneficial, cannot be accomplished without hazard. Functional change is beset with more risk than is physical change. The animal body is the most intricate, and also the most perfect, piece of mechanism known to man. It is a complex arrangement, made up of a combination of tissues and systems, all perfect and co-ordinated *per se*, yet so adjusted that they are interdependent, and, consequently, faults in one are often manifested in the other. We can see examples of this in our daily life. Since man assumed the upright posture his feet and knees have become the seat of troubles which rarely occurred in his simian ancestors. Since the natural angle of the knee has been lost the semilunar cartilages have become the seat of many traumatic lesions, such as one encounters but rarely in the quadruped. As regards his feet, we all know how they may affect his general health, and it is a fact that metatarsal bones may be fractured in the simple act of marching.

When we consider the nervous, the endocrine, the circulatory and

alimentary systems the hazards are more significant and disease and dysfunction more common and more difficult to explain.

We must try to know more about the normal working of the various parts of the body before we can presume to explain correctly or attempt to relieve the aberrations. It is unfortunate that the discoveries of Pasteur, Lister, and others, who initiated the conception of the germ theory of disease, retarded the progression of knowledge in other ways. For about 60 years, as a consequence of these discoveries, the bacteriologists held sway, for it seemed that the explanation of all disease or dysfunction of the body was in sight. A germ had to be found, and the optical instrument makers waxed rich. One must not detract in any way from the value of bacteriology to medicine, but, except for the few who, to put it mildly, were looked upon as unorthodox or cranks, all other methods of investigation were overshadowed.

One can see the effect of this in the review of the work on endometritis and dysfunctions of the female genital system. In spite of the striking empirical observations of the early workers, and also of the practices of certain primitive pastoral people who obtained some beneficial results following stimulation of the lower genital tract, the work of most scientific workers has been obsessed by the search for some causal organism. If this had not been the case, a follow-up of the stimulation theory might have opened the door at an earlier date to a study of the endocrine system. Whatever may be the cause of genital troubles, certain facts are now obvious. One of the most potent is that the endocrine system is so intricate, and so delicately co-ordinated, that you cannot call upon one section to do more than its normal work without upsetting the balance of the whole orchestra. If there had been no thousand-gallon cows the present anxiety about infertility would never have existed; certainly, it would not have achieved its present-day proportions and economic importance.

Nature cannot be abused without inviting repercussion.

Diseases of the central nervous system are also to the fore. We hear of new diseases, including degenerations and dysfunctions. Mostly, they are not new; it is simply an increase in incidence which has focused attention on them. In man they are supposed to be associated in some way with the " civilised " way of life. The same statement may apply to the domestic animals.

There may be a lot of truth in this, for man and animal do not live a natural life. Their feed is very different from that which nature intended, and it is recognised that diet plays a great part in promoting health and in resistance to disease.

There is not the slightest doubt that diseases and dysfunctions of the nervous system of dogs are much more prevalent nowadays than was the case only a few years ago. This increase in incidence may be another manifestation of reaction to environment, or of the working of so-called " natural law." It may be that in those cases where there is a viable causal factor this living thing is simply the old one under a new guise, and, being baulked in its customary mode of life or action, it may be taking advantage of " natural law " and reacting to new environment and circumstances.

# GENERAL ARTICLES

## EXAMINATION OF BULL SEMEN WITH THE ORDINARY AND PHASE CONTRAST MICROSCOPES

By P. G. D. MORRIS, M.A., M.R.C.V.S.,
*Department of Anatomy, Royal (Dick) Veterinary College, Edinburgh.*

### Introduction

A CONSIDERABLE number of samples of bull semen have been sent to this department for microscopical examination. These examinations were conducted on stained films and fresh, unstained material, using the ordinary and phase contrast microscopes. It was noticed that the anterior portion of the head of the sperms exhibited certain morphological variations. Therefore it was decided to examine a number of samples of semen from bulls of good fertility to ascertain whether these variations were normal.

It was known that the fragility of sperms necessitated careful preparation for their microscopical examination, and a careful technique had to be evolved to reduce the risk of creating artefacts.

### Materials and Methods

Samples of semen were obtained from a number of bulls of good fertility, most of the samples being from artificial insemination centres. Some of the work was carried out on fresh semen at an artificial insemination centre, and the remainder of the samples were received through the post, in small, tightly-stoppered bottles. The tubes were packed in contact with a strong rubber balloon containing ice and wrapped in cotton-wool. The samples were not diluted with egg yolk buffer solution, as this interfered with the microscopical examination.

Sperm counts were conducted on semen diluted 1 in 200 with saline and counted with a Metz hæmocytometer.

*Method for stained films*:—

(a) The semen was diluted with distilled water (one or two drops of semen to 3-4 c.c. of distilled water).

(b) A small drop of diluted semen was placed on one end of a clean slide and spread out by using the ground glass end of another slide. The semen was drawn behind the spreader. No pressure was put on the spreader, and care was taken that it rested evenly on the surface of the slide on which the smear was being made. Too much pressure resulted in the heads of some sperms being bent over, and even detached.

(c) The smear was allowed to dry.

(d) Fixed with methyl alcohol for five minutes.

(e) Stained with the following stains:—

Ziehl's carbol fuchsin and aniline gentian violet.
Heidenhain's iron hæmatoxylin.
Giemsa.

Of these methods, Heidenhain's hæmatoxylin gave the best results.

*Treatment of semen for examination with the phase contrast miscroscope.*

The semen was diluted with isotonic saline (one or two drops of semen to 3-4 c.c. saline). A drop of diluted semen was placed on a slide and covered with a No. I coverslip. The sperms seemed undamaged by the dilution, but the examination had to be done quickly, as changes occurred if the saline was allowed to evaporate and become hypertonic, with consequent distortion, which appeared to be confined to the head cap, as the acrosome membrane became crenated. Some degree of crenation of the acrosome membrane occurred in all untreated samples, but hypertonic saline made it more evident.

## Results

Examination of stained smears with the ordinary microscope showed the head of the normal sperm to be of a flattened, oval shape, with the anterior extremity covered by a distinct galea capitis. In smears stained by Heidenhain's iron hæmatoxylin, many of the sperms showed a narrow light zone between the anterior portion of the acrosome membrane and the nuclear material (Fig. I). In a small number of sperms, immediately beneath the most anterior portion of the membrane there was a narrow, finely granular zone, which stained deeply with Heidenhain's iron hæmatoxylin; this granular zone was separated from the nuclear material by the narrow light zone (Fig. I). Other sperms showed the zone between the galea capitis and the anterior end of the nucleus to be a diffuse grey colour. In nearly all sperms stained with Giemsa there was a deeply staining, narrow, granular zone immediately behind the acrosome membrane; this zone was noted to be more extensive than the granular zone demonstrated in some sperms stained by Heidenhain's iron hæmatoxylin, also the most deeply stained areas of this zone appear at the sides (Fig. II).

In a smear lightly stained with Heidenhain's iron hæmatoxylin the complete outline of the posterior limit of the galea capitis (Fig. I) could be seen, with, immediately behind it, a light zone limited posteriorly by the darker stained posterior nuclear cap. The caudal limit of the head was bounded by a darkly stained disc with a lighter stained centre, apparently continuous with the short and very lightly stained neck (Fig. I).

The extent of the galea capitis was more clearly defined in smears stained with Giemsa (Fig. II), and at the posterior limit of the head there appeared to be two small granules situated at the posterio-lateral boundary of the head (Fig. II).

The significance of these granules was not clear, and while they may be definite entities, it is realised that the question of an artefact cannot be entirely ruled out.

The connecting piece stained deeply with Heidenhain's iron hæmatoxylin; it had a beaded appearance when stained lightly by this stain or by Ziehl's carbol fuchsin and aniline gentian violet. In some preparations a narrow, clear "mantle" covering the connecting piece was seen; this was thought to be a thin layer of cytoplasm, but the central filament, as described by Rao and Hart (1948),[1] was not demonstrated by the staining methods used. At

the anterior end of the connecting piece there was a darkly stained limiting rod or disc, and a similar structure at the posterior end.

In stained smears the tail was clearly divided into two portions, the chief and the terminal filament. The chief piece was of great length. It consisted of a continuation of the axial filament, tapering fairly steeply caudally and surrounded by a thin, protoplasmic sheath, which ended abruptly, leaving the terminal short portion of the tail as a naked axial filament.

*Immature forms.*

Only a small percentage of immature sperms were observed in stained smears examined with the ordinary microscope. (See Table I.)

Smears made from the epididymis were examined, and it was found that nearly all the sperms in the head of the duct showed a cytoplasmic droplet attached to the neck region, whilst sperms from the tail of the duct showed a droplet at the caudal end of the connecting piece, and in some of the sperms from this region it was absent.

*Abnormal sperms.*

The types of abnormal sperms found were:—

(1) Abnormal heads—variation in size, shape and staining reaction.
(2) Abnormal neck and connecting piece.
(3) Abnormal tail.
(4) Tail-less sperms.
(5) Double heads and double tails.

### Phase Contrast Examination of Semen

With this method of examination the variations of the anterior portion of the galea capitis were pronounced, and three distinct types of sperm were noted:—

*Type I.* Sperms showing a dark zone immediately under the acrosome membrane and separated from the underlying nucleus by a narrow light zone (see Figs. III and IIIB). This type was found in large numbers in semen examined fresh at the artificial insemination centre, but in much smaller numbers in samples examined after transit through the post.

*Type II.* An intermediate type with the zone immediately below the acrosome membrane a diffuse grey; the percentage was small in both fresh semen and in samples received through the post.

*Type III.* The third type showed a light zone immediately below the acrosome membrane (Figs. IV and IIIA). This type was numerous in semen which was allowed to stand at room temperature for some time or passed through the post. In fresh semen a much smaller number was found.

Otherwise, except for this variation, the head in each type presented the same appearance. The cranial part was dark grey and the caudal limit of the galea capitis was clearly seen (Fig. IV); the caudal portion of the head was lighter, with the posterior pole appearing as a dark disc with a light centre (Fig. V). The neck, a short and narrow light zone, was bounded by two very fine dark lines, no other structures being visible (Fig. IV).

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## TABLE I.

| Sample No. | Sperms per c.c. of semen. | % Normal sperms. | % Immature. | Abnormals, including coiled tails. | Conception rate. | Breed. |
|---|---|---|---|---|---|---|
| I | 373,000,000 | 92.4 | 0.3 | 7.3 | 1.1 | Friesian. |
| II | 898,000,000 | 95.4 | 0.0 | 4.6 | 1.22 | Shorthorn. |
| III | 1,040,000,000 | 94.5 | 0.0 | 5.5 | — | — |
| IV | 430,000,000 | 97.0 | 0.0 | 3.0 | 2.0 | Shorthorn. |
| V | 356,000,000 | 86.5 | 0.0 | 13.4 | 2.4 | Friesian. |
| VI | 330,000,000 | 96.5 | 0.0 | 3.5 | 1.4 | Friesian. |
| VII | 792,000,000 | 93.0 | 0.0 | 7.0 | 1.1 | Shorthorn. |
| VIII | 1,600,000,000 | 92.5 | 0.0 | 7.5 | 1.9 | Guernsey. |
| IX | 430,000,000 | 93.0 | 0.0 | 7.0 | 1.8 | Guernsey. |
| X | 1,470,000,000 | 94.0 | 0.0 | 6.0 | 1.9 | Guernsey. |
| XII | 1,990,000,000 | 94.0 | 0.0 | 6.0 | — | — |
| XI | 1,550,000,000 | 92.5 | 0.0 | 7.5 | — | — |

Results of microscopical examination of semen from twelve bulls of good fertility. Stained smears and the ordinary miscroscope were used for examination of these samples. In the above Table the degree of motility is not included, because of the varying delay in receiving the samples. The figure-of-eight coiled tails were included as abnormals.

The body, or connecting piece, appeared very dark, but with careful focusing it was possible to see that the connecting piece contained granules; the cranial limit was marked by a dark rod or ring, and the caudal limit was also in the form of a dark rod or ring.

The chief piece appeared dark, but not so dark as the connecting piece, and in some cases a segmentation of this portion was observed. The terminal filament appeared as a short, dark, finely tapering thread.

*Immature forms.*

With the phase contrast microscope, the cytoplasmic droplets were more readily noticed than in stained smears. They were visible as clear, circular bodies containing some material which appeared very dark under the phase contrast microscope. This dark material was eccentric (Fig. VI). Large numbers of the cytoplasmic droplets were seen free in the seminal fluid.

*Tables.*

The results of microscopical examination of semen from twelve bulls of good fertility are shown in *Table I*. The counts of normal and abnormal sperms were made from stained smears with the ordinary microscope. A considerable variation in the number of sperms per c.c. was found, and possibly this is a breed variation.

The complete ejaculates were not available, so the total number of sperms for each complete ejaculate could not be ascertained.

*Table II* shows the results of sperm counts with the phase contrast microscope on unstained material.

## Discussion

*Technique.*

Various methods for making the smears were tried, and several different fixatives (formalin, heat, Flemming without acetic) were used, but undoubtedly the method given at the beginning of this paper yielded the most satisfactory results.

Treatment of the unfixed smears with 1 per cent chloramine for five minutes to remove the secretion from the accessory glands was tried, and although the resulting preparation was free from any background, the appearance of the galea capitis was changed.

Dilution of the semen with isotonic saline before making the film was also tried, but caused damage to the galea capitis, probably due to the great increase in concentration of the salt while the smear was drying.

Examination of semen with the phase contrast microscope has several great advantages. These advantages lie in the fact that no fixation or staining is necessary, that no smear need be made, and that the sperms may be examined alive in the untreated samples. The density of the sperms, however, in a good sample is too great for critical examination of individual sperms, therefore it was found necessary to dilute the semen with isotonic saline. This, as stated, does not damage the sperms, unless the saline becomes hypertonic.

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## TABLE II.

| NORMAL SPERMS. | | | | | | ABNORMAL SPERMS. | | |
|---|---|---|---|---|---|---|---|---|
| Samples. | Type I. | Type II. | Type III. | Immatures I. | Immatures II. | Coiled Tails. | Other Abnormals. | REMARKS. |
| A–H Fresh samples. I–K Samples sent through the post. | Dark zone immediately under anterior portion of the galea capitis separated from the nucleus by a clear zone. | Diffuse grey zone immediately under anterior portion of the galea capitis. | Light zone between anterior portion of galea capitis and nucleus. | Cytoplasmic droplet situated at cranial end of the body. | Cytoplasmic droplet situated at caudal end of the body. | | | |
| A | 34% | 13% | 45% | 5% | 3% | Not counted | Not counted | |
| B | 37% | 3% | 46% | 0% | 6% | 7% | 1% | |
| C | 47% | 15% | 36% | 0% | 1% | 0% | 1% | |
| D | 30% | 4% | 57% | 0% | 1% | 8% | 0% | |
| E | 66% | 11% | 18% | 1% | 3% | 1% | 0% | |
| F | 32% | 4% | 52% | 1% | 0% | 10% | 1% | In this count the figure-of-eight coiled tails had been included as abnormals. |
| G | 34% | 10% | 52% | 1% | 3% | Not counted | Not counted | |
| H | 27% | 14% | 52% | 1% | 1% | 2% | 3% | |
| I | 1% | 0.5% | 74% | 0.5% | 0% | 6% | 18% | Same bull as H, but sample sent through the post. Many of the abnormals were tailless sperms. |
| J | 0% | 2% | 80% | 0% | 1% | 9% | 8% | Same bull as A, but sent through the post. |
| K | 0% | 1.5% | 85.5% | 0% | 4% | 4.5% | 4.5% | Same bull as C, but sent through the post. Figure-of-eight coiled tails included as abnormals. One-third of the other abnormals were tailless sperms. |

The above table shows the distribution of sperms as shown by the phase contrast microscope in samples of semen from bulls of good fertility.

PLATE I

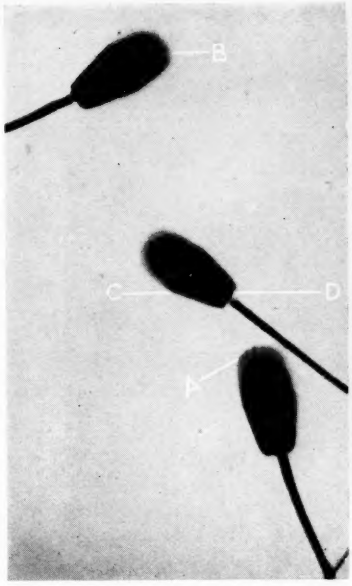


FIG. I.—Stained Heidenhains Iron Hæmatoxylin.
(A) Narrow granular zone.
(B) Light zone.
(C) Posterior limit of galea capitis.
(D) Lightly stained neck.
X 1,500 approx.

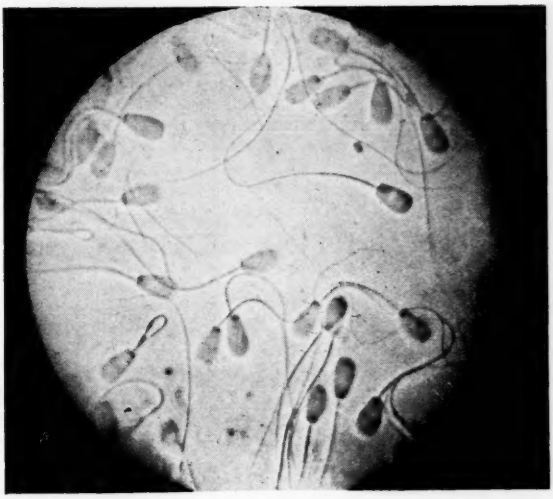

FIG. IIIA.
Phase Contrast. Unstained—Types I and III.
X 700 approx.

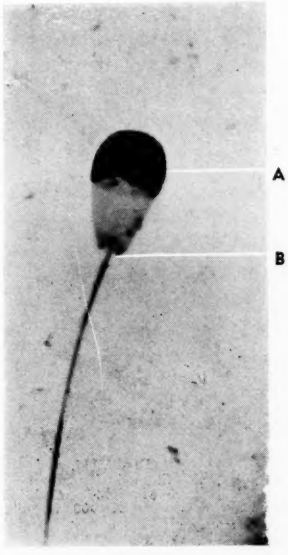


FIG. II.—Stained Giemsa.
(A) Deeply stained areas at side of head.
(B) Small granules.
X 2,000 approx.

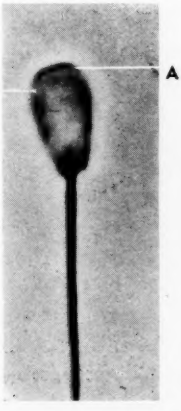


FIG. III.
Phase Contrast.
Unstained Type I.
(A) Dark zone.
(B) Light zone.
X 1,650 approx.

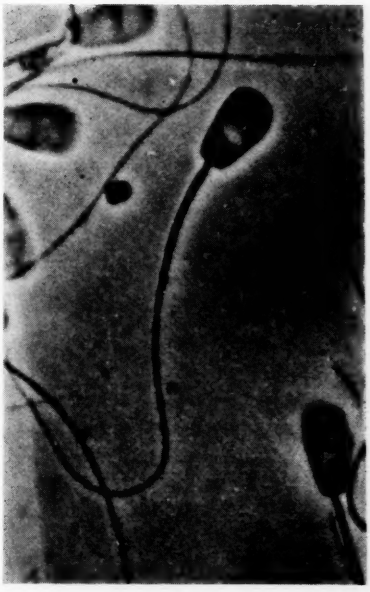

FIG. IIIB.
Type I Sperm (enlarged from Fig. IIIA.)
X 1,300 approx.

(*Article by Morris, page* 85)

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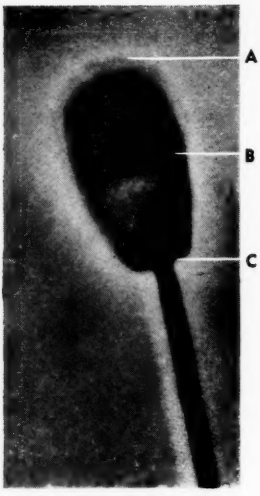


FIG. IV.
Phase Contrast.
Unstained—Type III.
(A) Light zone.
(B) Caudal limit of galea capitis.
(C) Neck.
X 3,250 approx.

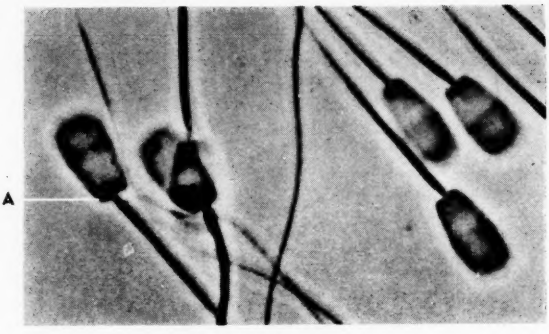


FIG. V.—Phase Contrast. Unstained.
(A) Dark disc with light centre.
X 1,500 approx.

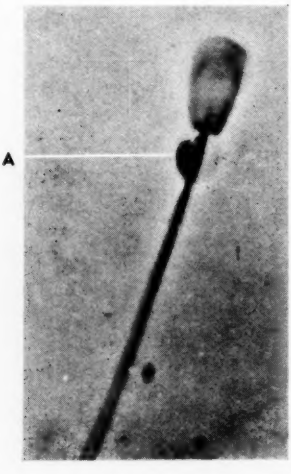


FIG. VI.
Phase Contrast. Unstained.
Immature sperm.
(A) Cytoplasmic droplet.
X 1,500 approx.

FIG. VII.
Phase Contrast. Unstained.
'Figure of eight' coiled tail.
(A) Dark zone.
X 3,250 approx.

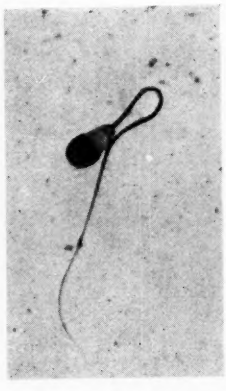

FIG. VIII.
Stained Giemsa.
'Figure of eight' coiled tail.
X 900 approx.

*(Article by Morris, page 85)*

*Findings.*

It has been shown that the galea capitis of the normal sperm of bulls may vary in appearance. Three types of variation have been noted when examining unstained semen with the phase contrast microscope.

*Type I.* Behind the most anterior portion of the acrosome membrane was a dark zone, immediately separated from the nuclear material by a narrow, clear zone.

*Type II.* The area between the most anterior portion of the acrosome membrane and the nuclear material appeared diffuse grey.

*Type III.* The above area was demonstrated as a clear zone by phase contrast.

Type I was found in large numbers in semen examined fresh at the artificial insemination centre, but in samples examined after transit through the post a much smaller number was observed. Many of the sperms with tails coiled in a figure " 8 " (Figs. VII and VIII) showed the dark zone of Type I in samples that had been kept some time after ejaculation. The sperms showing a figure-of-eight coiling of the tail were not counted as abnormal, as it was thought that this type resulted from shock (temperature) received immediately after ejaculation, since, according to Rao and Hart (1948),[1] " sperm containing the drop at the distal end of the connecting piece will show straight tails on ejaculation. If stored for one day or less at extremes of temperature (45 degrees F.), a high percentage will develop a bent tail around the drop, and this seriously interferes with motility and, presumably, fertilising ability. This bending of the tail will also be manifested in a certain percentage of cells without the protoplasmic drop. This is all evidence of the need for care in evaluating semen samples on morphology . . . ." " The appearance of bent-tailed sperm evidently resulted from too sudden exposure of semen to extremes of high or low temperature. The former may occur in the artificial vagina."

In many cases in sperms of this type, a cytoplasmic droplet can be seen in the loop of the tail; it seems possible that any abnormally large deviation of temperature may have prevented the sperms from casting off the cytoplasmic droplet after ejaculation. These sperms appeared to be very long lived; they showed motility long after the normal sperms, possibly due to their being unable to move about freely, and therefore not using up their energy at the same rate as the normal sperms. Perhaps the dark zone, as seen in Type I sperms, was only visible in sperms which had not used up their energy, and Types II and III were sperms motile for some time, and in consequence had used up their store of energy. It will be seen from Table II that the percentage of the different types (Types I, II and III) varied considerably, even in fresh samples of semen, due probably to a number of factors:—

(1) Temperature of surrounding atmosphere.

(2) Interval between ejaculations; if there were long intervals it is possible that sperms stored in the epididymis had " aged " by gradually using up their store of energy. So far, work has not been carried out to see what influence

the above factors have on the percentage of sperms Types I, II and III found in samples of normal semen, but it is hoped that such an experiment may be carried out in the future.

Blom (1945)[2] indicated that the galea capitis may become completely detached, and the numbers of sperms in which the galea has become detached greatly increased in animals with very long periods of rest between services, suggesting that degeneration of the sperm takes place in the epididymis, and that these changes are manifested by a loosening and complete detachment of the galea capitis of some of the sperms.

There are also the findings of Green (1940), who, working with rams' semen, using aceto-carmine stain and dark field illumination, according to Anderson (1945),[8] "observed a small hyaline vesicular structure contained in the membrane of the anterior border of some cells. The number of vesicles present in fresh or stored samples varied between rams and among samples from a given ram. The number of vesicles decreased with ageing and frequent ejaculation. . . ." "Green states that, because of physical differences, the anterior vesiculated region may play a more or less dynamic rôle in phenomena such as osmotic changes, permeability, or possible mechanical or enzymatic action associated with fertilisation. Samples having a larger percentage of vesicles are more desirable than other samples, because of the correlations found with certain physiological conditions of the sperm."

No similar vesicles were noted in bull sperms.

## Summary

Examination of semen with the phase contrast microscope reduced the risk of artefacts (such as may appear in fixed stained preparations).

Morphological variations of the anterior part of the head immediately beneath the galea capitis have been noted, and an attempt made to classify them as follows:

*Type I*—showing a dark zone immediately under the anterior portion of the limiting membrane of the galea capitis and separated from the nuclear material of the head by a narrow light zone.

*Type II*—a diffuse grey zone immediately below the anterior portion of the limiting membrane.

*Type III*—a clear zone between the membrane of the galea capitis and the cranial extremity of the nucleus.

## Acknowledgments

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# EXPERIMENTAL ALLERGIC ENCEPHALITIS AND IMPLICATIONS REGARDING THE ÆTIOLOGY OF DEMYELINATING DISEASES OF MAN AND ANIMALS: A Review

By J. R. M. INNES,*

THIRTY years ago, the ætiology of the demyelinating diseases had not aroused real active interest amongst experimental pathologists. This need occasion little surprise, for indeed, the pathology of many types had not yet been established. Subsequent recognition of an increasing number of clinico-pathological complexes in man, in which the demyelination might be a common bond, gradually focused attention on what is now one of the most important problems in neurology. There was some time-lag between medical and veterinary observations, but forms of demyelinating diseases have been identified in a wide variety of species—monkeys, dogs, horses, lambs, goats, and in some captive wild animals such as tiger bear and leopard—in many parts of the world. This aspect of comparative pathology is dealt with in papers on spontaneous disorders by King and Meehan (1948), Innes (1949), and in the monograph by Scherer (1944).

In medical neurology there are many authorities who believe that all demyelinating diseases are interrelated, and possible variants of a single pathological condition in which the myelin sheaths are primarily affected, with a lesser and lagging degeneration of axones, and rarely with accompanying necrosis of other neural and glial elements. For those interested in these views, reference might be made to Ferraro (1937 and 1944); he considers that many of the clinicopathological differences might be reconciled with the time of onset of the disease, the initial intensity of action of any causal agent, and the subsequent tempo of course. He veers to the belief that there has been a tendency to create new clinical and pathological subdivisions too easily, to insufficient discrimination of what constitutes acute and chronic reaction, and to too much dogmatic opinion on inflammation versus degeneration.

* Present address: Microbiological Institute, National Institutes of Health, Bethesda, Maryland.

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The problem of demyelinating encephalitis in dogs has become one of some magnitude to the small animal practitioner, and the study by McIntyre, Trevan, and Montgomerie (1948) revitalised somewhat the previous apathetic interest which was influenced largely by dogma and traditional beliefs. This recent work emphasised the possible rôle of a virus — hitherto confused with distemper—in the ætiology of at least one form of demyelination in the dog, and which is part of a peculiar complex commonly known as hard-pad disease. There are, however, still other forms in dogs and other animals which remain unexplained on a satisfactory basis of ætiology, and an outstanding example is swayback.

This dissertation deals with progress which has been made in somewhat unrelated fields of experimental neuropathology and immunology. It may show that advances may come from studies by methods other than those of direct observation on natural diseases. The bleak outlook on the ætiology of demyelinating diseases of some fifteen years ago is much brighter. Some attention is given to how some of the experimental work originally arose, and how, although deductions therefrom are still in a conjectural phase, it might have some broad implication towards elucidation of the nature and cause of demyelinating diseases. It must certainly pave the way for clarification of the chemical mechanism of a process whereby, under certain conditions, myelin is selectively broken down and disappears, slowly progressively and in patches in some forms, and diffusely with great rapidity in others.

An experimental approach to the problem was a prime necessity for any real progress to be made. Consideration is omitted of the many studies made on the experimental production of demyelination by disparate types of chemical substances, e.g., carbon monoxide, cyanides, lead, saponin. Excellent reviews on this aspect are available in the papers of Weston Hurst (1944) and Ferraro (1944). From this mode of approach it was manifest that demyelination, as a kind of lesion, could be produced by toxic materials of a diverse nature, which in itself might not be surprising, any more than the appearance of liver cirrhosis following experimental hepatic damage by various means. As Hurst stressed, all this seemed to indicate that "there was no common determinant more narrowly specific than injury to white matter at some time or other." This reinforced the belief of many that there might be multiple causes of demyelination, rather than a common one, and we might cite here the mysterious causal rôle of copper in swayback with the contrast of a virus in hard pad. We are not concerned with the potential rôle of viruses in demyelination, but perforce it might be mentioned that many workers have believed that viruses were not concerned in diseases with this lesion, and that in established forms of virus encephalitides demyelination was never found. These are views which may require some revision.

The history of neuroparalytic accidents following small-pox vaccination in man dates back to over 60 years ago, but more attention was concentrated on the disease by the epidemics which occurred shortly after 1920. Pathological studies by Turnbull (1912) (see Turnbull and McIntosh [1926]), and others

later, showed that demyelination was an outstanding characteristic lesion. The ætiology is just as obscure now as it was then; it is still undecided whether it is the primary virus, another virus made active in the nervous system by the vaccine, or some allergic phenomenon. The history of the occurrence of a parallel form of neuroparalysis subsequent to rabies vaccination is almost as old, for the first published observation was by Laveran (1891), according to Harvey and McKendrick (1908). As far back as that the latter workers considered that these "accidents" were probably initiated by the large amounts of foreign nervous tissue injected—a theme to which they returned (see Harvey and Acton [1923]). Since then many others have tackled the problem and, depending on their experimental results, have argued for, or against, the concept that the nervous disorder was due to (a) a modification of the original rabies virus infection, (b) some other virus, or toxin, potentiated by the injection, or (c) some sensitisation effect produced by the inoculation of foreign nervous tissue. All the important references on these concepts can be found in the papers by Stuart and Krikorian (1928), Bassoe and Grinker (1930), Weston Hurst (1932), and Jervis, Burkhart and Koprowski (1949). The first-mentioned authors described demyelination in human cases of such accidents, and deemed it unnecessary to seek for any explanation other than that the nervous tissue injected contained some deleterious substance besides actual vaccine. They produced paralysis in rabbits by the injection of normal homologous and heterologous brain material alike, and this did not depend on the quantity given, but on some unknown quality which could be inactivated by phenol. They did not observe demyelination in the rabbits, but they mentioned the presence of a "myelitis." Weston Hurst (1932) (in a valuable analysis of the literature on post-vaccinal encephalitis, as well as recording results of personal investigations) reported paresis in rabbits after subcutaneous, or intramuscular, injections of saline suspensions of human brain, but also did not observe demyelination. He affirmed then that there was insufficient evidence which would support contentions regarding the presence of some demyelinating factor in normal nervous substance, but he broached the question whether or not the injection might stimulate some latent factors. (This paper should be read along with the review of Hurst [1944].) The idea that the original rabies virus infection in neuroparalytic accidents was involved in some modified form can be regarded as obsolete; cases have been recorded in individuals who were inoculated after being bitten by dogs found later to be non-rabid. Further, we know that the rabies virus does not cause demyelination under any conditions, but, in contrast, produces definite lesions in the grey matter, including the pathognomonic inclusion bodies (Negri bodies) in neurones. Finally, no one has ever succeeded in isolating by animal passage any infectious agent from the brains of human cases of post-vaccinal encephalitis (rabies or small-pox), and, as will be noted later, nor again from comparable canine ones after rabies vaccination.

An identical type of demyelinating encephalitis may occur in dogs after rabies vaccination, although adequate reports are very few and many merely record the occurrence of clinical paralysis. The first paper to describe lesions,

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but without specifically mentioning demyelination, was that by von Mócsy (1938); (1938); he stated the incidence of this complication in vaccinated dogs was about 0.2 per cent in Budapesth. The only good neuropathological study is that by Jervis, Burkhart and Koprowski (1949), in the U.S.A. In this latter country the importance of rabies from the public health standpoint, and the wide use of prophylactic vaccine, has indubitably resulted in concentrated interest. In spite of the few reports in the literature, it is believed that many cases occur in dogs which are either ignored, are not examined and reported, or they may be confused clinically with other forms of nervous disorder. Jervis, *et al.*, described four cases in Great Pyrenees out of a total of 60 of that breed and 30 St. Bernards; all dogs had been vaccinated by a single subcutaneous injection of suspension of horse-brain tissue infected with rabbit-brain fixed strain of virus inactivated by phenol and heat. The four dogs developed neurological signs after 14-20 days, and showed paralysis of an ascending type, tremors, myoclonia, but no fever. The animals were killed for autopsy within a few days after onset of illness. Attempts to isolate any infectious agent (rabies or any other virus), from the brains of these cases by inoculation into mice or dogs were negative, which is in line with all previous results obtained in the case of human post-vaccinal disease. The lesions were comparable to those seen in human neuroparalytic cases, and the canine condition was labelled "acute multiple demyelinating encephalomyelitis." Pertinent comments were also made on the similarity of the lesions to those seen in experimental monkeys which had been injected with brain emulsion plus adjuvants, as well as to those in certain spontaneous diseases of dogs. Patchy demyelination, perivascular in character but widespread throughout the whole white matter, perivascular infiltrations, microglial reaction with formation of scavenging cells (phagocytes, macrophages, compound granular corpuscles), and incipient macroglial proliferation were all present. The problem of ætiology was discussed, particularly in the light of work on experimental allergic encephalitis, and which is dealt with below. It is important, at this stage to stress that apparently no worker, as yet, has tried to produce demyelination in dogs by this technique, or by the use of "rabies vaccine" prepared in the usual way but minus the actual virus; this is, in fact, a crucial experiment which remains to be done. (Jervis and Koprowski (1949) have also recorded subacute demyelination in mice after repeated intraperitoneal injections of rabies vaccine.)

The fact that antirabic vaccine (but not the virus) was somehow a predisposing cause of at least one type of post-vaccinal demyelination, no doubt was responsible for ideas engendered that spontaneous demyelinating diseases might be due to some allergic effect. Conjecture along these lines was prominent many years before experimental work was undertaken. Hurst (1944) gives an excellent précis, but it is only necessary to mention certain relevant observations which have led to more recent experimental work. A number of workers had produced brain specific antisera by injecting rabbits with suspensions of heterologous brain tissue. The demonstration of brain specific antigen bolstered

the concept that perhaps the post-infective demyelinating encephalitides might, in some manner, be associated with the development of specific antibodies for white matter. Rivers and his colleagues (1933-1935) demonstrated that repeated intramuscular injections of heterologous brain emulsions, prepared in various ways, into monkeys caused in most of them (in some after a long latent period), ataxia, facial paralysis, and sometimes blindness; these symptoms were linked with severe demyelinating encephalitis, especially affecting the pons, cerebellum and periventricular regions. As Rivers acknowledged at that time, the mechanism of production of these lesions might seem to be far removed from any which might be present naturally, but a means for experimentally induced demyelination was available and capable of further elaborate study. Confirmation that progressive demyelination could be produced in this way came from Ferraro and Jervis (1940), who speculated further on the ætiology of spontaneous degenerative disorders, and that they might be associated with the formation of autogenous antibodies against the tissue affected. (This was in line with other work, starting with Masugi [1933], on the production of glomerulonephritis in animals by injecting anti-kidney serum.) Schwenkter and Rivers (1934) had already shown that complement fixing antibodies were produced in rabbits by injection of emulsions of homologous brain which had been allowed to stand for 5-30 days. Specific brain antigen was six times as abundant in white matter as in the grey, was absent in fœtal or new-born rabbits, increased with maturity, and thus ran parallel with myelin formation.

Some workers were not successful in repeating this work of Rivers, *et al.* Innes (1935), while working on swayback on lambs, administered suspensions of sterile rabbit brain (ground up in Locke's solution, with alcohol added) thrice weekly to pregnant ewes in the first place, and then to the lambs born, for over a year without any clinical ill-effect whatsoever. Kolb and Bolton (1940) were unable to produce lesions in the brains of rats by injection of rabbit serum containing brain antibodies over a period of three months. Hurst (1944) obtained negative results in monkeys injected with alcohol-ether extracts of rabbits' brain, or in sheep using pig-brain inoculum. In the light of more recent work, a number of explanations might be put forward for these apparent failures; the most ready one, as far as my own work was concerned, might be bound up with the technique of preparation of the brain emulsions used. From what is known now, the "allergic factor" is present in the brain of all mammals, and can produce demyelination under certain conditions of experiment, but it may be alcohol soluble (see Alvord [1949]).

An outstanding advance in our knowledge of experimental demyelinating diseases arose by work which was carried out originally by Kabat, Wolf and Bezer (1946-49) and simultaneously and independently by Morgan (1947). This type of experimental approach would appear to have taken root from observations which had been made in an entirely unrelated field of immunology and serology. Lewis and Loomis (1926) had studied the effect of chronic infections on the formation of specific antibodies, and had shown that antibody titres to substances not related to tubercle bacilli were higher in tuberculous guinea-pigs

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than in controls. Dienes and Schoenheit (1929) showed when egg white, or horse serum, was injected into a tuberculous focus the sensitisation to these antigens was much more vigorous, and the same enhanced effect could be produced by dead tubercle bacilli. The direct lead arose from the work of Freund and his colleagues (see papers from 1937 onwards). In a paper in 1937 they quoted that a synergistic effect of various substances upon immunisations had been known for a long time, and cited a paper by Swift and Schulz (1930). In their initial experiments, the addition of a small quantity of paraffin oil to heat-killed tubercle bacilli intensified the reaction conspicuously and persistently, and promoted sensitisation and antibody formation in both guinea-pigs and rabbits. When this inoculum was injected subcutaneously, dead tubercle bacilli, amidst characteristic tubercular lesions, soon appeared in regional lymph nodes and the lungs, but did not occur in the absence of paraffin. Many other papers by Freund have elaborated the idea that antibodies at higher titres could be produced by incorporating the antigen into a fatty substance with heat-killed tubercle bacilli—the so-called adjuvants. This was responsible for arousing the attention of Kabat, *et al.*, and Morgan to the possibility that autosensitisation, or autoantibody formation, might be a mechanism in the causation of certain demyelinating diseases, and, perhaps, with particular reference to post-vaccinal encephalitis. In the original paper by Kabat, Wolf and Bezer (1946), sterile normal rabbit, or monkey, brain was homogenised, paraffin and heat-killed tubercle bacilli were added, and then autoclaved at 60 degrees C. to destroy autolytic enzymes. In the earlier work of Rivers, the number of injections of brain emulsions necessary to produce paralysis was from 30-100, and intervals of 3-13 months elapsed before any symptoms appeared. With the technique used by Kabat, *et al.*, the number of the injections was reduced to 1-3, and the time interval to 17-60 days. Signs in experimental monkeys included trunk ataxia, weakness of limbs, rotation and retraction of head, ptosis, strabismus, tremors and reduction in vision. The lesions were restricted to the CNS, and resembled those described earlier by Rivers. Focal, and perivascular, areas of demyelination were observed in the brain stem, pons, cerebellum and cerebrum, with fewer and less intense areas in the cord, and occasionally in the optic nerves. There was an initial perivascular infiltration of polymorphonuclears superseded by lymphocytes, and hæmorrhage or thrombosis was rare. Microglial proliferation and mild astrocytosis were seen, but the axones were well preserved and apparently undiminished in numbers, in spite of myelin loss. The demyelinated foci were nearly always related to a venule, vein, capillary, arteriole, or artery, in that order of frequency. In many monkeys no gross lesions could be detected, and there were never any changes in the peripheral nerves or retina. Blood cultures were sterile, and inoculation of cerebral tissue from affected monkeys into other monkeys and rabbits had no ill-effect. In their paper of 1947 (Wolf, *et al.*), much attention was given to a comparison of the pathology with that of spontaneous conditions; the occasional punched out areas of myelin loss were thought to be reminiscent of some phases of disseminated sclerosis, while the sparing of the U-fibres and the more diffuse lesions were

rather like diffuse sclerosis in children. They considered that the whole syndrome resembled disseminated encephalomyelitis and sclerosis in man, but the course was considerably shortened. The symptom-complex in both was dependent on the predilection of the pathological process for certain structures in the nervous system, while remissions and relapses occurred in both simian and human conditions. Later, they showed that the reaction was specifically limited to the use of adult brain, but of any mammal; it could not, for example, be produced by emulsions of rabbit lung made in the same way, nor of fœtal rabbit brain, monkey kidney, peripheral nerves, or by adjuvants alone. The antigen was organ-specific, for species such as chick brain would produce the experimental disease, but not the brain from frog or fish.

In a series of other experiments they produced the same severe nervous disorder in a monkey by emulsion made from its own brain, previously removed surgically (frontal lobectomy) and then injected after the monkey recovered. The responsible factor was essentially, or predominantly, present in white matter, for fœtal brain was innocuous until myelin appeared (see also earlier work of Rivers); it was resistant to formalin fixation, to heating and boiling, to treatment by ultrasound, was destroyed by alcohol (both extract and residue), and it was not present in peripheral nerves. It was thus possible to postulate with some experimental support that an antibody to injected brain tissue reacted with the nervous system to produce demyelination. They admitted there were obvious gaps in the links of evidence, and the main one was the unsuccessful attempt to transfer the disease with serum or cellular exudates, from affected monkeys, or with serum from rabbits injected with brain emulsion. It was possible, however, according to them, that the hypothetical antibody might be continuously removed from the circulation. The use of adjuvants provoked conjecture, for it was shown that not only were they necessary, but they had to be present at the same site of inoculation of brain tissue. Emulsion of brain without acid-fast bacilli injected on one side, and only bacilli (heat killed) on the other side of the body of the same animal failed to result in nervous disease. Alvord (1949) contended that the bacilli were only necessary to produce the accelerated form of demyelination. The function of the bacilli, according to Kabat, *et al.*, might be to initiate an epithelioid cell reaction in close proximity to the antigen.

Many other workers have now made comparable studies, using other species of animals, and have confirmed and amplified the original observations of Kabat, Wolf and Bezer, and of Morgan. Ferraro and Cazullo (1948) also produced the disease in monkeys, while Freund, Stern and Pisani (1947), Morrison (1947), and Jervis and Koprowski (1948) studied the experimental condition in rabbits and guinea-pigs. Alvord (1949) also used guinea-pigs, and made many timely critical comments on the extent to which experimental evidence could be harmonised with facts concerning spontaneous demyelinating diseases of man and animals. In another paper, Alvord, *et al.* (1948), showed that pyribenzamine protected guinea-pigs against the effects of histamine and anaphylaxis, but did not prevent " allergic encephalitis " following the use of brain emulsion plus

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adjuvants. This suggested to these workers that histamine played no part in the pathogenesis unless it acted in a manner not influenced by anti-histamine drugs, as does occasionally happen, for example, in the case of histamine-induced gastric ulcer. Good, *et al.* (1949), have made a very interesting observation which requires substantiation, but which might be of the utmost importance; this concerned the use of para-amino benzoic acid combined with salicylates, which was said to reduce the incidence of allergic encephalitis in guinea-pigs from 90 to 10 per cent if the animals were premedicated.

All this work on experimental demyelination has gradually built up a clear-cut disease entity which may yet be shown to have some true allergic foundation along the lines speculated on by so many. The manner in which the disease is produced experimentally may appear at first sight to be highly artificial, and the sequence of events of autosensitisation which might lead to spontaneous demyelination remains unknown.

Some of the investigators have remarked on the presence of eosinophils, and epithelioid-like histiocytes in the nervous lesions, and have compared this with those seen in the Arthus phenomenon. Hurst (1944) gives much attention to this. Davidoff, *et al.* (1932), for example, showed that the lesion of the Arthus phenomenon could be produced in guinea-pigs first sensitised extra-cerebrally to various antigens and then injected intra-cerebrally with homologous antigen. A severe Arthus-like manifestation was also seen in monkeys sensitised the same way by Kopeloff, *et al.* (1936), but, unfortunately, no histological examinations were made of the brains at that time; these experiments were repeated much later by Ferraro, Kopeloff and Kopeloff (1941), who showed that this Arthus phenomenon, at the site of intracerebral inoculation of antigen (egg white) in sensitised monkeys embraced the presence of small foci of demyelination. They suggested that, in the pathogenesis of this type of lesion, the damaged tissue in combination with the foreign protein produced brain-specific antibodies.

As a consequence to this experimental work in the U.S.A., there have now appeared a number of analytical papers in which there are serious suggestions that the ætiology of spontaneous demyelinating diseases of man may be associated with some auto-brain antigen-antibody (isoallergic) reaction. Reference can be made to the papers by Ferraro (1944), and to Stevenson and Alvord (1947).

It is clear that all this work of the past 15 years, starting with that of Rivers, has resulted in a reorientation of ideas on the ætiology of demyelinating diseases in man and animals. There are good reasons to believe that the experimental techniques which have been evolved may give the lead to further clarification of diseases such as disseminated sclerosis, post-vaccinal encephalitis, and a host of other apparently related nervous disorders. Manifestly the next step must be in a chemical direction to isolate the factor in white matter which has a pathogenic property against the same tissue. In this direction Bell, Wright and Habel (1949) have published a preliminary

report on work which emanated out of experiments designed to purify rabies vaccine. By a process of extraction with calcium acetate solution this vaccine may be freed from the factor which causes allergic encephalitis. It is not removed by extraction with benzene and ether and appears to be water soluble, which in itself is a considerable advance. Alvord (1949) stated that this factor is a heat-stable phosphatid-like substance and does not resemble any previously described brain haptens except possibly the alcohol-soluble one.

This experimental approach to the problem of demyelination is not without veterinary importance. In the first place anti-rabic vaccine encephalitis may be a much more serious disease in the U.S.A. and other countries than has been suspected. Britain has been kept free from rabies since 1926 because of quarantine regulations and consequently there need be little vaccination of dogs except of those being exported abroad. Until methods are available, and adopted, for removal of the allergic factor in rabies vaccine, then any practitioner who vaccinates a dog should clearly bear in mind the consequences if, in a matter of a few weeks, the dog becomes progressively paralysed.

Starting with the study by McIntyre *et al.*, there has been an awakening of the urgency for clarification of the nature and cause of encephalitic syndromes in dogs, some of which may be due to a virus, or viruses, which are distinct from distemper. There appears to be one demyelinating disease in dogs associated with a distemper-like illness and hyperkeratosis of the pads which is caused by a virus; there are perhaps other forms of demyelination which are not transmissible to the ferret or the dog, which were referred to in the paper by McIntyre, *et al.* (1948). If an infectious agent cannot be incriminated then it behoves us to note progress which is being made in studies of experimental allergic encephalitis. Further, in any experimental work with dogs, or indeed any of the laboratory animals, in which inoculum of brain tissues is used to identify infectious agents it would be as well to be aware of the potential dangers residual in nervous tissue itself. It is advantageous also to recall that swayback is a demyelinating disorder of lambs which seems to be prevented by the administration of copper to the pregnant ewes; there has been no contribution worth one iota since that fact was established which has furthered to any advances on pathogenesis.

Weipers (1949) in a tabulation of various virus infections of dogs, compiled from many original sources, suggested the possibility of the occurrence of an encephalitis following Dunkin-Laidlaw distemper vaccination; he listed cellular infiltrations and glial clusters in the pathology. The latter was quite unjustifiable for no observer has indeed seen any such lesions. In spite of the millions of dogs which have been vaccinated since the method was initiated no reports of this complication have reached the literature, and in the face of existing evidence this possibility would seem remote. It might be imagined that any suspected cases of this nature, if they did occur, would have been readily noticed, for in the case of post-vaccinal encephalitis of both man and dog (rabies and smallpox) the onset of nervous disorder is characterised by its regular, relatively short, time interval after the vaccination. This is also

a characteristic of the so-called post-infective demyelinating encephalitides in man which may follow in the train of various exanthematous infections. The nature of the inoculum in distemper-vaccine-virus might also preclude the possibility that it has demyelinating potencies. Post-vaccinal encephalitis might be rarely expected to follow louping-ill vaccination in sheep because of the nervous tissue in the vaccine: no reports have been made. (Allergic encephalitis has been produced in sheep by sheep brain emulsion by the author.)

## Addendum.

Since the above paper was written a few other publications have appeared which are of importance and amplify the statements already made. Lumsden, C. E. (1949), *Brain*, 72, 198, deals with very comprehensive studies of the experimental disease in guinea-pigs; he showed that the demyelinating lesion has a definite period of incidence with a constant peak around the 32nd day; it is produced only by nervous tissue and not at all by adjuvants alone, nor by adjuvants in conjunction with kidney, liver and muscle at equal concentrations. This confirms the findings of other workers, but Lumsden found that the lesion *can* be initiated by sterile peripheral nerve tissue which had not been done before; this is important for it was somewhat of a puzzling nature to consider that the demyelinating factor was present in the myelin of the brain but was absent in the peripheral nerves. He observed one peculiar feature in that rat brain was apparently innocuous to guinea-pigs and vice versa, but the brain of the latter could produce nervous disease in rabbits. Lumsden considered that the analogy between this form of experimental demyelination and human "post-exanthem encephalitides" was too close to be insignificant. If this resemblance means anything then the lesions of the latter may be due to some chemical factor arising from the skin rash (for we know it is not due to the original virus) and closely allied to that agency, not yet identified chemically, present in normal mammalian white matter. The same idea might be applied to theorising about the ætiology of post-vaccinial encephalitis in man. Olitsky, P. K., and Yager, R. H. (1949), *Jl. Exper. Med.*, *90*, 213, produced disseminated encephalomyelitis like that seen in monkeys and guinea-pigs in a strain of Swiss mice by injection of normal mouse brain with adjuvants; acute, subacute and chronic forms, could be produced, and they suggested consequently that the mouse might be the animal of choice for further work on this problem of isolating the causal factor in white matter. Cazullo, C. L., and Ferraro, A. (1949), *Arch. Path. 48*, 316, showed that an acute encephalomyelitis could be produced by injecting guinea-pigs with homologous guinea-pig brain intra-peritoneally. The nutritional state of the animals was important, and there was a general drop in body weight with the appearance of the first symptoms of hypotonia and asthenia evidenced by poor righting reflexes; later signs included ataxia, tremors, convulsions and paralysis—flaccid at first, and later spastic. Peers, J. H. (1950), *Amer. V. Clin. Path.*, has contributed a highly critical review with particular reference to a comparison of the pathology with that of human post-vaccinial encephalitis.

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# BOVINE ENDOMETRITIS—A REVIEW OF LITERATURE TO 1947, WITH SPECIAL REFERENCE TO THE CATARRHAL TYPE OF THE DISEASE*

By F. L. M. DAWSON, M.R.C.V.S., M.A., Ph.D., B.Sc.,
*Ministry of Agriculture and Fisheries.*

It has for some time been known that the catarrhal form is only one of those which chronic endometritis in the cow can assume; lines of demarcation are not sharp, and this condition merges with the less severe form, lacking a continuous discharge on the one hand, and, on the other, with the more serious condition of pyometra, when large quantities of slime and pus accumulate in the uterus, owing to failure or inadequacy of the discharging process. For this reason all the forms must to some extent be considered together; especially must the catarrhal type be linked with pyometra, for both are more distinctly established clinical entities than the "simple" form, and may be shown to differ from one another, not in kind, but only in degree. The problems which these conditions present, and the published researches which have been done on them, can be considered from a number of points of view, but the early workers engaged most actively in controversy on the aspect of ætiology, to which that of therapy was closely linked. The therapeutic-ætiological viewpoint is therefore the best to adopt in reviewing the development of ideas on the subject.

### Aspect of Ætiology with Therapeutics

Interest in endometritis was first aroused in Switzerland. Therapy by the insertion of a creosote-soaked plug into the cervix was described in the *Schweizer Archiv fur Tierheilkunde* in 1843; Durler, in 1856 (quoted by Andres[2]), was a pioneer in cervical dilatation and evacuation of pus by mechanical means. An ecbolic used in human medicine, Juniper tea by mouth, was also given, with the same end in view, about this time. From about 1875, the practice of manual expulsion of the corpus luteum became quite general among Swiss veterinarians. All this work was, of course, purely empirical, based on the fact that spontaneous recovery cases were observed always to evacuate any purulent content. By 1890, the growing acceptance of the germ theory of disease led Ehrhardt to introduce the practice of disinfectant uterine irrigations by catheter (he killed several cows with strong solutions!), and Wapf, 1897, claimed good results with this technique, using dilute alum. His work brings to an end what may be termed the *early experimental period.*

### First Controversial Period: 1900-1924

The work of Zschokke, published in 1900, and quoted by Albrechtsen,[1] is a conspicuous milestone in the historical highway. Zschokke was a very fine clinical observer with great breadth of view, and anticipated the results of the most recent research to a remarkable extent. He noted a connection between endometritis and prolonged anœstrus, and that in this latter condition a persistent corpus luteum could accompany both catarrhal endometritis and

* An article introductory to a clinical study of the condition, undertaken by the writer.

pyometra. The performance of 110 autopsies on cows with cystic ovarian disease revealed to him that most frequently uterine lesions were absent; he therefore suggested that this disease must be due to the failure of some principle which caused the rupture of a ripe follicle in the normal cow, an amazingly close approximation to the well-established 1950 view that it is caused by a deficiency of pituitary luteinising hormone! His routine treatments, based on these observations, consisted of cyst-rupture and corpus luteum expulsion, with cervical dilatation in addition. He did also make limited use of weak disinfectant uterine washes, following the line indicated by Wapf. Hess, writing in 1905, seems to have been a disciple of Zschokke, and went a step further, renouncing irrigation, especially as it often involved rather brutally forcing a catheter through a closed cervix, which he considered very bad practice. He recognised the "uterotropic" effect of corpus luteum expulsion even more clearly than Zschokke, and stated explicitly that the uterine disease was secondary to a primary condition of ovarian abnormality; for treatment, he employed only manual methods, ovarian manipulation as practised by Zschokke, supplemented by general massaging of these organs and of the uterus per rectum.

In Hess's publication year, the first work out of Switzerland was begun by Albrechtsen in Denmark. He did not publish his results until 1917, but their appearance marked the establishment of a Danish-German school at bitter variance with the Swiss one, even though another Dane, Martin Poulsen, writing about 1910, found his local observations put him in complete agreement with Hess. Albrechtsen [1] thought that all endometrites were caused by a primary microbe infection in the uterus, either blood-borne or picked up via the vagina off the standings; rarely it might be venereal in origin. This was to have a secondary effect on the ovaries through infection ascending to them; though he practised plugging the cervix with iodine tincture wads, "as an adjuvant," treatment was essentially an attempt to *disinfect* the tract with moderate to weak solutions of iodine through a catheter; later variations were the use of enormous quantities of very weak solutions up to thirty litres, and electrolysis by filling the uterus with copper sulphate and then inserting electrodes. Such innovations, well publicised, were profitable, if strenuous, for Albrechtsen, for no other operator could handle such large volumes for irrigations (Ramvad [18]): Albrechtsen devised also the valuable technique of cervical retraction right into the vulva, and had no hesitation in subjecting this organ to drastic expansion measures with scissors and large-bore catheters. In this respect, too, he was in direct opposition to Hess, but laid more stress on his "primary uterine infection" theory, which conflicted very sharply with the latter's views, which Albrechtsen directly attacks in his "reasons why ovarian changes are secondary to uterine infection." These are, that ovarian cysts and retained corpora lutea are not seen uncomplicated by endometritis; that endometritis exists at first for weeks and months without cysts, which, however, have a strong tendency to eventual development; and that on curing the endometritis by the application of treatment aimed at disinfection, the ovarian condition resolves.

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Albrechtsen obtained very good clinical results, and his views were largely accepted by the Germans, Richter (1920) and Oppermann (1922). Richter devised a classification of endometritis, into "first degree," simple; "second degree," catarrhal; and "third degree," pyometra; which is still very useful. Oppermann introduced the practice of preparing vaccines from bacteria of the vaginal flora, to use for endometritis prophylaxis and treatment; this was, of course, a direct sequence from the pathogenetic views of Albrechtsen. The influence of the junior, Danish-German, school was dominant over that of the senior, Swiss, school during the next period, 1925-1935, of research and development round this question.

### The Rise of "Nielsenism": 1925-35

Folmer Nielsen, also a Dane, was in most respects a follower of Albrechtsen, but his work, seen in historical perspective, inaugurates a new and higher stage of development in approach to and knowledge of the subject. Before considering this, it is appropriate briefly to survey contributions by three other authors, who all adhere strictly to the doctrine of the primary nature of uterine infection, with, consequently, irrigant disinfection as the only treatment of avail. In fact, these three, Quinlan, Stader, and Jaeger, are "strict primitive Albrechtsenites"; although publishing in the later period when Nielsenism was in the ascendant. Quinlan,[17] working in South Africa, applied Albrechtsen's treatment recommendations, and his paper, 1929, repeats them with acknowledgment almost work for word, together with the "reasons why pathological ovarian changes are secondary to uterine infection." It therefore seems probable that these methods gave satisfaction, though the treatment trials are not detailed. He lays more stress than his teacher on the point that *corpus luteum retention* is secondary to an accompanying endometritis, "though expulsion may appear to have a good effect on endometritis in the *slight* cases," and makes his own attack on Hess, "whose hypothesis appears very unlikely." Stader,[22] 1932, in the United States of America, declared himself solidly in support of Albrechtsen's ætiological views, but devised treatment improvements, an ingenious technique for uterine disinfection by 24 irrigations given hourly in succession, with sodium hypochlorite; this demanded much less operative skill than Albrechtsen's own methods, and only a single professional visit. Stader's attack on Hess is the most impassioned of all. Jaeger,[9] a German, in 1934 carried out a useful bacteriological contents analysis on pyometra cases, terming all the organisms he found "the agents of the disease," and recommending as the best treatment tried a mechanical emptying, followed by disinfection with 1 per cent chinosol.

Nielsen, at the Dublin Congress of 1926, was the first to introduce the treatment of endometritis to the notice of the veterinary profession of Great Britain (to this day the Albrechtsen type catheter is exclusively known here as a "Nielsen catheter"); he took the same view as the older Dane that uterine infection was a primary lesion, and again repeated the "reasons why ovarian involvement must be secondary." He considered that the persistence of the

corpus luteum in endometritis was not functional, but purely morphological and owing to intoxication, from organisms in the uterus, delaying its regression.

However, in contrast to Albrechtsen and his immediate adherents, Nielsen laid a heavy stress on the fact that ovarian cyclic impairment is common in the more severe degrees of endometritis (though attributing this result, again, to bacterial intoxication). He was the first to note that the iodine irrigation treatment appeared to have some effect in reinitiating a lapsed cycle, and appreciated that such irrigations owed part of their therapeutic value to this property (despite his insistence that their main mode of action was disinfectant). Neilsen's second cardinal difference from Albrechtsen lay in his attitude to the views of Hess. While according these no great sympathy, he admitted that corpus luteum expulsion and other ovarian manipulations were effective treatments, approaching in usefulness, he implies (though not too closely), the uterine irrigations. This contrasts abruptly with Albrechtsen, who eventually abandoned such a method of treatment altogether; but Nielsen was sufficiently of Albrechtsen's school to feel at a loss to account for such good results from ovarian manipulations. His final therapeutic conclusions are in favour of iodine irrigations as the agent of first choice, with ovarian treatment as an important adjuvant. (He also tried Rivanol, an acridine derivative, and found it as effective as iodine.)

In the next few years a number of "Nielsenists" adopted these views, and published variations on the same theme. Küst, of Giessen, proved himself a whole-hearted Nielsenist, laying stress on the susceptibility of "all types of uterine infections to the same therapeutic disinfectant measures"[12] (1932), while advocating corpus luteum expulsion as important in pyometra[13] (1934), and describing the use of a course of copaia balsam and turpentine by mouth to render a difficult corpus luteum more amenable to the operation. Küst was also the first of the moderns to return to the ecbolic idea; in contrast to the early Swiss with juniper tea, he gave his ecbolic—carbamyl choline—by injection. This, with the other measures, gave good recovery figures. Ramvad,[18] in 1935, tried pituitrin as an ecbolic, concluding it was a valuable routine prophylactic, but uneconomical in treatment.

Baumann[4] (1933), working near Berlin, used colloidal silver for his disinfectant irrigations, noting a high incidence of ovarian pathology in his endometritis cases and employing manipulation freely in its treatment, without attempting to assess the relative values of the two different methods. Kuipers[11] (1935), in Holland, recognised more clearly still than Nielsen the usefulness of iodine irrigations in reinitiating an œstrus cycle; he thought it helpful to irrigate, 24 hours previously, with a half-saturated salt solution in order to dissolve mucus, so permitting the iodine, in his own words, to "*werk dieper in*"!

Two essentially Nielsenist authors neither expressed their views nor recorded their experiences until well into the next "historical period." Lörcher[14] (1937) carried out a study similar to that of Baumann; for disinfectant he used sodium

perborate, talc, and a foam producer, which could be carried about in powder form—more convenient, as he claimed—and dissolved on the farm. Schaetz,[21] as late as 1944, alleged that pyometra was primarily due to direct infection with one of three specific organisms; cyclic impairment was a quite important secondary feature, and *ovarian hormone,* available by this time, was admitted to be a useful aid in treatment, though disinfectants were to remain the chief stand-by.

### Impact of Reproductive Hormone Discoveries: 1935 Onwards

Firstly, "Conscious Irritationism": Riedel (quoted by Andres[2]), in 1935, sounded a challenging note when he declared he held no theory as to the true nature of disease in the "first degree" cases of Richter's classification; he was not convinced that bacterial infection was ever necessarily present. Iodine irrigations gave good results in his hands, and he was convinced they acted not as disinfectants, but as counter-irritants, "shocking" the *vis medicatrix naturæ* of the animal into bringing about a cure. He was the first to advocate a final irrigation two days *post-coitum.* In forming these views, Riedel must have been influenced, even if unconsciously, by the newly-available knowledge (Zondek, 1931, and his successors) that the working of the reproductive system was governed largely by pituitary-secreted hormones, having their impact more directly on the ovary than on the uterus.

Hignett[7] (1940), the first British writer in this field, adhered closely to Nielsen's views on the primary nature of uterine infection, associating, as did the latter, invasion by vaginal flora with the effect of an original *Brucella abortus* infection. Hignett advocates exactly the same treatment methods, but differs rather profoundly from Nielsen, inclining towards Riedel, by his specific belief that the curative action of the iodine is counter-irritant only, not disinfectant. He thought its mode of action might be through effect on the innervation, but when he obtained a measure of proof for his views on the counter-irritant nature of iodine, he also showed that, whatever the mechanism, the treatment affected the reproductive endocrine system; for two anœstrus heifers, in which there was no suggestion of an infective condition or uterine abnormality, were speedily brought on heat by iodine irrigations. A further measure of proof for the value of counter-irritant therapy was obtained by Wille[24] (1942). He frankly set out to counter-irritate the cervix in endometritis cases, including catarrhal ones and those of pyometra, by spraying the organ with cantharides. Seventy-four per cent of 82 cases subsequently conceived quite quickly, having previously been sterile for periods certainly up to three months, and probably longer.

Höll[8] (1944) is also a "conscious irritationist"; he used iodine irrigations specifically for their "tissue-stimulant" effect, and not as a disinfectant. Furthermore, he was the first to combine conscious "stimulation" with "disinfectant" therapy, for he combined "surfen," a complex organic bacteriocide, of proved efficacy in some types of tissue, with his iodine in solution. Results appeared to be as good as might have been expected from the application of the two principles, 77.5 per cent of catarrhal cases recovering after a single treatment.

Wille having shown that cervical stimulation alone could appear to cure endometritis, and Hignett having proved local counter-irritation successful in inducing heat, topical interest attaches to the hypothesis which Hignett mentions, that the true effective action in iodine therapy is the mechanical stimulus to the cervical nerves produced by passing the catheter. If this were correct, it would account for the success of counter-irritation and mechanical dilatation of the cervix practised empirically as early as 1843, and 1856 (by Durler), and later by Albrechtsen, Nielsen, and Kuipers, who attributed the success of their treatments to disinfection. Albrechtsen characteristically attributed the success of dilatation merely to " the relief of stenosis," but his treatments usually included unconscious cervical stimulation, with tincture of iodine—by a curiously similar method to the creosote plug of 1843! Kuiper's recognition, even clearer than Nielsen's, of irrigation's efficacy in reinitiating ovarian activity, and of the importance this factor had in bringing about a cure, is a close parallel to Hignett's result with non-infected heifers. Thus, according to this hypothesis, many of the pre-1935 workers, firmly believing in iodine disinfection, were in reality obtaining their cures as " irritationists," even though unconscious ones, and so, in sharp contrast to the " conscious irritationists," Riedel, Hignett, Wille and Höll, who deliberately set out to produce an effect by the use of drugs for this particular purpose—cantharides, for example.

Oddly enough, another " unconscious irritationist " is found as late as 1941 in Andres,[2] who was the pioneer in the intra-uterine use of " Juniper tea," which he termed " metratonin." In one way Andres was a follower of Küst and Ramvad—the advocates of drugs to produce muscular contraction—for he considered the important factor in a cure was to obtain the maximum duration of the maximum degree of uterine tone; this his preparation did very efficiently. He differed from Küst and the other Nielsenists in believing *disinfection* to be of quite secondary importance, though subscribing to the view that organismal uterine infection was a primary lesion. In 50 per cent of his 500 treated cases, however, œstrus was induced and the cycle re-set within five days of the first treatment, showing that as well as a highly active muscle-tone producer, " metratonin " was a potent counter-irritant or tissue stimulant, similar in effect to Kuipers's and Hignett's iodine and to Wille's cantharides. But Andres was so absorbed in the aspect of tone-production that, although recording these results, he does not seem to appreciate their full significance, or their relationship to the work of the latter-named authors.

Secondly, reversion to the early Swiss view: Kostner[10] (1939), of Vienna, was the first to return, if only by implication, to the views of Hess that ovarian pathology was primary; for his sole treatment recommendation for pyometra was corpus luteum enucleation; he deprecated all direct uterine interference, and stated explicitly his belief, for which he had a certain amount of experimental justification, based on 18 cases, that purulent contents were produced primarily by autolysis, for example, of a dead fœtus; infection of the uterus from the vagina being purely a secondary consequence, from dissolution of the cervical mucous plug.

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A full and frank return to the Hess outlook was made by Rowson and Spriggs[19] in 1942, *with* the full support of modern endocrinology, but *without* reference to, or apparent knowledge of, the writings of Hess, which shows well how far these were ahead of their time. In 1942, Brownlee[5] had expressed a theory for the cow, based on a vast volume of recent experimental work on small laboratory animals and from many hands, that ovarian-type hormones (œstrogens) produced by the placenta at term caused regression of the corpus luteum, thereby allowing the "pituitary rhythms" (associated with pituitrin) to be felt by the uterus, which bring about parturition. Rowson and Spriggs stated, in conformity, that it was the persistent presence of a corpus luteum that governed pyometra, and recommended treatment by injection with the synthetic œstrogen stilbœstrol, which simulates the placental œstrogens of term, causing regression of the persistent corpus and thus sensitising the uterus to the pituitary rhythms. They claimed the advantages for their treatment over enucleation that it was easier, safer, and could be repeated, and recorded 15 case records to demonstrate its effectiveness. Moore, from America, in 1944, supplemented these with still more evidence of the high efficacy of this treatment. Recalling the views of Zschokke on the nature of cystic ovarian disease, so violently opposed to Albrechtsen's, and others, it is of interest to note that Hancock[6] (1947) published a series of observations on treatment of this condition by injecting preparations of pituitary luteinising hormones, showing, in the first place, that uterine pathology was not usually evident in these cases, and, in the second, that the treatment was apparently very effective in restoring a normal healthy cycle. In fact, Hancock's work confuted the Albrechtsen opinions and confirmed most strongly the conclusions drawn by Zschokke 47 years previously.

An isolated therapeutic school is that in favour of autohæmotherapy into the uterus, established on rather scanty protocols by Azbukhin and Yolkov[8] (1935) and Baldo Baldi, quoted by Quarante[16] (1939). Timoseeff, quoted by Andres,[2] advocated diathermy.

### Aspect of Bacteriology

The earliest workers were too preoccupied with disinfectant treatment to show much interest in the nature of organisms found associated with uterine pus. Küst, as late as 1932, expressed their views well when he said that "all types of agent were equally susceptible to chemotherapeutic measures." Albrechtsen briefly noted the recovery of streptococci twice from pus; Nielsen, in the only sentence he wrote on specific organisms, stated that this same genus might be significant. Quinlan had cultures made from 7 sets of fallopian tubes in endometritis cases, and found *Bacterium coli,* various types of cocci, and *Corynebacterium pyogenes,* all of which he thought might be equally significant. Baumann took a single set of cultures from each of 9 out of his 36 endometritis cases, and found the same organisms as Quinlan, though *C. pyogenes* was present in no less than 5; however, Baumann did not see any generic significance in this finding; no case seen impressed him as differing clinically from another —except, occasionally, in its outcome!

In 1933, the pathogenic significance of the protozoan parasite, *Trichomonas fœtus,* became widely recognised in Europe; in the literature previous to this date is is impossible to distinguish between cases due to this direct venereal infection and other cases, but by 1934, Küst noted that 71 per cent of 124 pyometra cases from which he made cultures were associated with this flagellate: and there is justification for the assertion that they were so *caused,* for the organism has since been shown to fulfil Koch's postulates. Tagliavini,[23] however, writing in the same year, was less well abreast of the latest developments when he stated that pyometra "is caused by benign bacteria from the vagina, the accumulating secretion being then sterilised by phagocytic activity, to the extent that it becomes an optimum medium for the multiplication of some flagellates"! Küst's 1934 observations are evidence of an increased interest in the clinical bacteriology of his cases; he noted that many non-Trichomonad cases contained mixed bacteria, whereas many others were apparently sterile: furthermore, he distinguished *C. pyogenes*—associated cases as a clinical entity apart from the others; his series included 5, "of which 4 were cured, showing that this type of case need not have so bad a prognosis as is generally supposed" —apparently a tradition of bad prognosis when *C. pyogenes* was present had been arising in Germany. Jaeger, again in 1934, further East in Germany, near Hanover, analysed bacteriologically 51 pyometra cases. He found 18 per cent sterile, no less than 25 per cent containing *C. pyogenes,* and the remainder Trichomonads, and cocci of various types: his treatment results with *C. pyogenes* differed from Küst's and confirmed the "tradition," for though his overall rate for subsequent conception was 80 per cent, in the Corynebacterial cases it was only 7 per cent—i.e., only one of thirteen cases conceived again, and the prognosis was more than 11 times worse when this organism was found present. However, Kostner, analysing 18 pyometra cases in 1939, found 9 of them sterile; the remainder only with cocci and *Bact. coli,* no *C. pyogenes* being found; this encouraged him towards the conclusion that the organisms discovered were unimportant secondary invaders, especially as they proved unable, as a rule, to kill mice on injection. The only bacteriological result that Andres, 1941, records is "sterility" in two pyometra cases; but Hignett, the previous year, fell in line with the tradition which Küst introduced to the literature, when he said he considered iodine irrigations yielded good results, "even when *C. pyogenes* is *the causal organism.*"—the last words involve a bold assumption. Runge[20] (1942), stated that pyometra cases were very common in Poland, and could yield any of four microbe types, *Brucella abortus, Bacterium coli,* cocci, and *C. pyogenes,* without particularly stressing any one of them; but Holl, in 1944, agreed with Küst, Jaeger, and Hignett that *C. pyogenes*-associated cases were a distinct clinical entity. Holl hoped to destroy, with surfen, staphylococci, streptococci, and *Bacterium coli*; but, in addition to 38 pyometra cases so treated, there were others in which, *C. pyogenes* having been found, he attempted no treatment, but advised slaughter. He was alone in condemning cases with β-hæmolytic streptococci in the same way, though Schaetz thought that "hæmolytic streptococci" with *C. pyogenes* were the sole types of causal bacteria, *Trichomonas fœtus* being the other possible agent.

### Aspects of Clinical Research Methods, and of Prognosis

It is obvious that the various methods adopted, and the circumstances in which the different workers were placed, have a bearing on the type of results obtained and the value that can be attached to them. Whereas not all authors gave sufficient data for forming a useful estimate of their research methods, none of them, apparently, made any attempt at controlling the effectiveness of treatment by leaving a proportion of cases to the unassisted *vis medicatrix naturæ*; though Nielsen had adequate normal healthy controls to demonstrate the prolongation of post-partum anœstrus in diseased cows. Data are very vague as to the length of time cases had existed before treatment was applied, and thus recovery percentages in the articles have really very little meaning. Albrechtsen and Nielsen were on contract work; Nielsen treated 206 catarrhal cases and had about 70 per cent "cures"; he worked on only 10 herds, thus being able to obtain a percentage-incidence, and visited 7 of these not more often than monthly, so that the average case probably received about three visits. Baumann, in contrast, was in private practice, so could afford to undertake treatment only in good types of risk—he would attempt no cases where the discharge was purely purulent, but only when a mucous discharge contained pus flakes. Perhaps significantly, conception rate for his 38 cases, visited usually twice or three times each, was 89 per cent. Küst had his cases sent in to an "obstetrical clinic"; he had 124 pyometras and about 250 catarrhal cases; his excellent opportunity for numerous serial examinations and for leaving controls untreated was largely wasted. Jaeger's pyometra study was quite broadly based on 86 cases; Lörcher had only 38 endometrites of all types; in general practice, he could only manage an average of two visits per case, and if a treatment failed the case could not be further followed. Holl treated 100 catarrhal cases; his was a travelling clinic, so there was a minimal chance to follow up cases, many being only seen once. He deplored again and again the shortage of anamnesis and of opportunity for serial examinations over a period.

On prognosis, a measure of agreement has been noted, that it is poor when *C. pyogenes* is isolated in numbers from the discharges; but in general there are wide disagreements. Richter, quoted by Quinlan, mentioned a 50 per cent recovery rate for catarrhal cases "under optimum conditions"—which must mean, when taken early and skilfully treated. But Lörcher found his recovery rate just as good when the cows had been barren for a year—however, his special circumstances make this statement suspect. Nielsen's figures prove a better prognosis than Richter's estimate, and are not inconsistent with Hignett's opinion that "prognosis is not at all favourable in neglected cases, but good results are obtained when the condition is tackled soon." (The most recent work indicates that Hignett's was probably a correct judgment.)

### The Literary Aspect: Summary and Conclusions

The language difficulty is one which can have played only a very small part in complicating the work of authors on this subject. For instance, the remarkable parallel between the work of Zschokke and Hess at the turn of the

century, and that of Rowson and Spriggs forty years later, has been discussed; the omission of reference by the English authors to the Swiss results must be ascribed to other than linguistic causes, for most of the latter are summarised in Albrechtsen's book, of which an English edition early appeared. An early issue of Nielsen's paper was in English (and hence, admittedly, his preponderating influence on the later English work, which was relatively small in amount), but otherwise nearly all the articles have been in German, and written by Germans, Swiss, or Danes. German translations soon appeared of certain papers, such as Albrechtsen's, which were originally in Danish, while it may be assumed, so great is the linguistic similarity, that any Dane could read German.

It is therefore the more surprising to find that most authors have laboured under the handicap of a decidedly imperfect knowledge of contemporary literature. Albrechtsen stated dogmatically that another Dane, K. Nielsen (not to be confused with Folmer Nielsen) was the first to express the corpus luteum; he was unaware, or chose to appear so, that the operation had been routinely performed in Switzerland several decades previously, and he apparently chose to ignore the "inconvenient" results obtained in Zschokke's autopsies on a series of nymphomaniacs, which conflicted with his own views. Baumann and Jaeger give imperfect lists of German references only, with the exception of, respectively, one and two papers in English which are quoted. Kostner's paper appears to be one of the most remarkable examples of what may follow from inadequate literary preparation. Five years before he published, Küst had given a clinical classification of pyometras, including two clearly distinguished divisions, *pyometra post-partum* and *pyometra post-conceptionem,* though he did not apply these actual terms; Meggio,[15] writing about the same time as Küst, went further, pointing out that prognosis is far more favourable in the *post-conceptionem* than *in the post-partum* form. Staub, quoted by Andres (1936), expands this point, and discusses the difference between these two clinical types in considerable detail. Yet Kostner, who could have had access to all these papers, does not recognise the existence of the *post-partum* form at all: his study is, in fact, a valuable contribution to knowledge of the *post-conceptionem* form, but he treats it as the only type existing—"pyometra" [*sic*] "arises from the death of the embryo, followed by autolysis." In this way his conclusions on the relative benignity of the disease as a whole are accounted for, as are what he considers the irreconcilable differences between his and the earlier work—he found, for instance, no histopathological lesions in the wall of the uterus. The essay by Andres has a copious bibliography, which is of unique value in its review of the early Swiss writings; it refers to the work of certainly one "conscious irritationist"—Riedel—but carries no appreciation of the significance of Riedel's school of thought, though such an appreciation would have enabled Andres to score an additional very telling point in favour of the drug he advocates—that it functioned not only as a muscle-tone producer, but also as a counter-irritant. Andres does, indeed, note in passing that a renewal of cycle was often induced by his "metratonin," but he fails to back this

observation with the full weight and militant force of the available " conscious irritationist " evidence on the value of this type of treatment.

Quinlan is outstanding as the only author who carried out a thorough literary preparation for his own experimental work; he considered all relevant papers up to his own publication date, but in his case the preparation might be considered overdone, so unquestioningly does he accept Albrechtsen's and Nielsen's views and reasoning. The possibility exists that he might have made more notable advances in the therapeutic-ætiological field had he approached his clinical research with a more open mind, and afterwards related his own provisional conclusions with those of the earlier workers in European countries.

A brief summary shows that chronic endometritis was treated empirically, in Switzerland, during the nineteenth century, chiefly by cervical stimulation and dilatation and by corpus luteum expulsion: Zschokke and Hess developed on these foundations, early in the twentieth century, a school of clinical thought which maintained at least the " pathological independence " of the ovary from the uterus; at furthest, the " pathological and physiological dependence " of the uterus on the ovary. (Hess was later misrepresented as having claimed that only ovarian disease, not uterine, was of *any* significance.) Treatment was therefore to be directed equally towards the two organs, or even mainly in the direction of the ovary. A Danish-German school arose, during the following 20 years, which was directly opposed and actively hostile to the Swiss school, claiming that the ovary was completely dependent, pathologically, upon the uterus, which became primarily involved in disease, by means of external infection with unspecified bacteria. A direct attack on the microbes in the uterus with bactericidal irrigations could therefore be the only treatment of avail. Albrechtsen was the chief protagonist in this conception, being followed, *inter alia*, by Quinlan and Stader. Nielsen, in 1925-26, inaugurated an advance when, although convinced of primary uterine infection, he admitted the significance of anœstrus in endometritis, and that the effect produced in re-initiating a cycle contributed to the value, mainly disinfectant, of iodine irrigations for treatment. His second " defection " from the strict Albrechtsen view lay in his admission that ovarian manipulation had a comparable therapeutic value with uterine irrigation. For the next ten years many supported this outlook, including Küst, and Kuipers, who more clearly recognised that an important effect of the iodine was its re-initiation of cycle. Following the discovery, in the general field, of the endocrine control of reproductive physiology, Riedel, in 1935, declared the effect of iodine was due to counter-irritation, not to disinfection, and, subsequently, Hignett, Wille, and Holl subscribed to this view, for which they obtained a measure of experimental proof; this, incidentally, demonstrated that the stimulant effect could evoke a rapid response from the reproductive endocrine system via the ovaries.

It is therefore suggested that the good clinical results obtained by the earlier protagonists of " disinfection " were really due to the counter-irritant effect, which the treatments were equally well calculated to produce. Almost con-

current with the Riedel school of " conscious irritation," none of whose adherents ever attempted to prove that iodine would not also disinfect, was the return to the original doctine of Hess. Kostner (1939) was a " neo-Hessian " only by implication, but Rowson and Spriggs (1942) stated frankly that treatment must be directed to influence the ovary in order that changes there might, in their turn, affect conditions in the uterus. They explained this view in terms of the most recent endocrinology, and justified it by a number of case records.

Bacteriologically, very limited observations previous to 1933 contributed to a view that of a number of floral organisms, each was equally significant. In that year the epizootic significance of the protozoan infection Trichomoniasis was realised. Küst, in 1934, mentioned it as a preponderating cause; he also mentioned parenthetically a small group of 5 cases associated with *C. pyogenes*, and an apparent tradition that prognosis is bad where this germ is present. Jaeger, the same year, corroborated this, adducing 13 such cases, of which 1 recovered; and, finally, Holl found 9 cases with either *C. pyogenes* or β-hæmolytic streptococci, in which he considered prognosis automatically so bad that treatment was not worth attempting. Other streptococci, staphylococci, and *Bacterium coli* were considered causes of a type of endometritis responsive to treatment. No other author considers the β-hæmolytic streptococcus to constitute a distinct clinical entity, but Hignett (1940), though quoting no protocols, makes a fourth who believes prognosis to be much worse in cases associated with *C. pyogenes*, which he assumes, with Schaetz, who makes a fifth, and with Jaeger, to be " the causal organism." Schaetz thought Trichomonads and both α- and β-streptococci were the other organisms to produce clinically similar effects.

No study carried out on endometritis included an attempt to assess the true value of therapy by leaving a proportion of cases as untreated controls; numbers of visits to individual cows, and, consequently, serial examinations in time, were few, averaging not more than three per case. Failures to respond to treatment were rarely followed up to the slaughter house or to eventual recovery despite treatment; and no study possessed balance over all aspects; those helpful bacteriologically, for example, such as Jaeger's, being poor or non-existent autopsically; a good autopsical study, such as Quinlan made, was correspondingly poor on the clinical side. These many deficiencies were eloquent of the wide scope for future work on the subject.

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# THE CONTROL OF EQUINE STRONGYLOSIS AND PERIODIC OPHTHALMIA WITH A RATION-ADDITIVE

By A. H. QUIN, D.V.M.,
*Kansas City, Missouri, U.S.A.*

In every country where horses are raised, sclerostomiasis, or strongylosis and periodic ophthalmia, or moon blindness, are afflictions of major importance. But, in view of research developments this past decade, the statement can be made that both sclerostomiasis and moon blindness may be added to the list of preventable diseases.

Dimock[1], and, later, Todd, Hansen, Kelly and Wyant[2], have published reports of controlled experiments which demonstrate that an optimum daily intake of 30 grains of phenothiazine per horse per day will effectively reduce *in vivo* infection with strongyles and prevent reinfection, as evidenced by the number of strongyle eggs passed by treated horses. Further, it has been well demonstrated that a fractional daily dose of phenothiazine markedly damages the fertility of the small nematode eggs.

Conversely, the classical experimental reports of Jones, Maurer and Roby[3], of the U.S. Army Veterinary Corps, conclusively prove that a daily intake of 40 milligrams of riboflavine (vitamin $B_2$) per horse effectively prevents the occurrence of periodic ophthalmia. This observation has now been confirmed by veterinarians working under field conditions throughout America, and is rated as one of the epochal veterinary research discoveries of the present century.

## A Field Test

A group of 16 thoroughbred yearlings, at Woolford Farm, near Kansas City, was made available for a practical field test. This famous stud lists such noted sires as Lawrin, 1938 Kentucky Derby winner, Blue d'Or, Historian, Maida, and others. The 16 yearlings had daily access to blue grass pastures heavily infected with strongyle eggs and larvæ. Fæcal counts indicated an average strongyle egg count of over 3,000 eggs per gram of fæces. The yearlings were placed on a special formula, designed to:—

1. Control strongyle larvæ.
2. Prevent periodic ophthalmia.

Brood mares with foals at side. Woolford Stud Farm near Kansas City, Missouri, U.S.A. The foals are kept free from strongyle larvæ by small daily doses of phenothiazine.

(*Article by Quin, page* 116)

3. Insure against vitamin D deficiency, trace element deficiencies, and to supplement the Ca : P balance.

The ration-additive so prepared contained, in each tablespoonful, the following ingredients :—

| | |
|---|---|
| Phenothiazine ... ... ... ... | 30 grains. |
| Riboflavin ... ... ... ... ... | 40 milligrams. |
| Vitamin D ... ... ... ... ... | 1,000 I.U. |
| Potassium Iodide ... ... ... ... | 10,000 P.P.M. |
| Manganese Chloride ... ... ... | 100 P.P.M. |
| Copper Sulphate ... ... ... ... | 100 P.P.M. |
| Cobalt Sulphate ... ... ... ... | 100 P.P.M. |
| Iron Sulphate ... ... ... ... | 10,000 P.P.M. |
| Calcium Phosphate and Sweetened Base ... | q.s. |

### Procedure

Each yearling was given one tablespoonful of the aforecited formula in the daily grain ration for twenty-one days. Medication was then withheld for seven days, and repeated for three weeks. This cycle of medication was maintained, for the purpose of the test, over a thirteen-week period.

### Results

At the end of three weeks after institution of medication, a five-ball dung sample was collected from each yearling. Strongyle eggs were counted according to the technique outlined by Stoll[4]. Egg counts from the yearlings showed an average 69 per cent reduction at the end of this three-week treatment period.

Dung samples were again collected at the ninth and thirteenth week of treatment. The results are shown in the accompanying chart. It will be noted that 12 head of the yearlings are completely negative for strongyle eggs. The remaining 4 head show egg counts of 333 or less per gram of fæces. It is generally conceded that levels within this bracket indicate subclinical endoparasitism so far as the small nematodes are concerned.

It was also noted that the ascarid egg counts remained unchanged during the period of observation. We concur with Dimock, *et al.*, that fractional daily doses of phenothiazine are ineffective as an ascaricide.

### Other Observations

Inspection of the 16 thoroughbred yearlings four months after initiation of the medicated ration-additive reveals exceptional growth—a few 16 hands at the withers—an exceptionally even hair coat, clear skin, and no bony deformities of any kind. There have been no cases of moon blindness among the adult stock on the farm.

### Summary

A ration-additive supplement containing 30 grains of phenothiazine and 40 milligrams of riboflavin effectively controls strongylosis and moon blindness of horses. The dosage scheme is carried on for three weeks; the drugs are

withheld for one week, and medication is maintained on this cycle throughout the year. A 2 gram daily dose of phenothiazine did not reduce the ascarid egg count.

**Acknowledgments**

The special formula reported in this field test was supplied by Jensen-Salsbery Laboratories as Ribophene-D. We gratefully acknowledge the co-operation of the Woolford Thoroughbred Farm, Kansas City, Missouri.

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Reduction in sclerostome egg count after ninth and thirteenth week following fractional daily dose of phenothiazine. See accompanying article for details.

| Name of Horse. | June 25, 1949 (Ninth week). | July 25, 1949 (Thirteenth week). | Remarks. |
|---|---|---|---|
| Larado* ... ... | 66 per gram | 0 per gram | Free from eggs. |
| Logical ... ... | 133 per gram | 0 per gram | Free from eggs. |
| Sylvestra (colt) ... | 0 per gram | 0 per gram | Free from eggs. |
| Insco Lassie* ... | 866 per gram | 200 per gram | Marked reduction. |
| Bohemian Lass ... | 66 per gram | 0 per gram | Free from eggs. |
| Iggy (colt) ... ... | 0 per gram | 66 per gram | Slight increase. |
| Even Stitch ... ... | 0 per gram | 0 per gram | Free from eggs. |
| Wise Niece (filly) ... | 200 per gram | 0 per gram | Free from eggs. |
| Heartening (filly) ... | 0 per gram | 0 per gram | Free from eggs. |
| Up Swing (colt) ... | 0 per gram | 0 per gram | Free from eggs. |
| Vice Versa (colt)* ... | 200 per gram | 333 per gram | Slight increase. |
| Achieve (colt)* ... | 66 per gram | 133 per gram | Slight increase. |
| Mixed Emotions* ... | 66 per gram | 0 per gram | Free from eggs. |
| Versatility ... ... | 0 per gram | 0 per gram | Free from eggs. |
| Scarlet Insco ... | 133 per gram | 0 per gram | Free from eggs. |
| Better Be Good ... | 0 per gram | 0 per gram | Free from eggs. |

* Ascarid eggs present.

Note: Egg counts below 200 per gram are considered as negative under practical conditions.

# STUDIES ON THE BLOOD OF DOGS
## IV.—Hæmatological Findings in Dogs Suffering from Some Common Diseases: (a) Pneumonia; (b) Distemper

By M. R. El HINDAWY, M.V.Sc.(Cairo)
*Lecturer in Pathology, School of Veterinary Medicine*
*Fouad 1 University, Cairo, Egypt*

### (a) Pneumonia

This affection, as met with in 26 dogs examined, occurred mainly during winter, associated with cold, damp and changeable temperature, though in some cases it followed as a secondary complication to distemper. In most, however, there was no indication of distemper and the pneumonia was ushered in merely by symptoms of more or less severe nasal catarrh, such as are now generally recognised to arise in dogs quite apart from a preliminary true distemper infection. Depression and restlessness were constant symptoms, with usually a high temperature, accelerated pulse and short, rapid, shallow and abdominal respirations, dyspnœa being a pronounced feature, with a short painful cough in most cases. Severe cases terminated fatally.

Post-mortem lesions of advanced broncho-pneumonia were noticed usually in both lungs, though in some cases they were confined to one lung, and, in a few cases, only a single lobe or a portion of a lobe was affected. Pleurisy with serous effusion in the chest cavity was encountered in some animals.

The blood picture in these cases was as follows, the data being recorded separately for the severe cases,[10] which terminated fatally, and the mild cases[16] which recovered but in which well-marked lesions of pneumonia were detected at post-mortem.

*Red Blood Corpuscles*

These were markedly decreased in number. The red cell count ranged from 2,300,000 to 6,310,000 per c.mm. in the severe, fatal cases, and from 2,850,000 to 7,610,000 per c.mm. in the milder cases, the average being 4,700,000 and 4,860,000 per c.mm. respectively.

*Hæmoglobin*

This ranged between 15 and 66 per cent in fatal cases and between 22 and 72 per cent in mild cases; the average, which was markedly decreased, amounted to 44 and 50 per cent, respectively.

*Colour Index*

This varied from 0.13 to 0.56 in severe cases and from 0.24 to 0.70 in mild cases, the average figure for both groups being 0.45. This is markedly below normal.

*Reticulocytes*

These were increased in number; they ranged from 0 to 8 per cent of the erythrocytes in severe cases and from 0 to 16 per cent in mild cases. An average of 1.7 for the former and 1.9 per cent for the latter was recorded.

*Total Leucocyte Count*

A great variation was noted. A marked leucopenia was recorded in grave

cases and a marked leucocytosis was found in mild cases. The leucocyte count ranged from 4,300 to 9,800 per c.mm. for the former and from 10,200 to 25,200 per c.mm. for the latter, the average count being 7,230 and 14,356, respectively.

### Differential Count

*Neutrophiles*

Neutrophilia was marked in grave cases and was slighter in mild cases. The neutrophile percentage of the total leucocytes ranged between 52.2 and 94.7 in the former cases with an average of 77.8. In the latter cases, the neutrophile percentage ranged between 57.4 and 92.3, with an average of 72.8.

*Eosinophiles*

These cells were totally absent in some cases in both groups. In other cases they were markedly reduced in number and percentage. The count percentage ranged between 0 and 10 in grave cases, with an average of 2.3 of the total leucocytes, and between 0 and 20, with an average of 4.6 of the total leucocytes for milder cases.

*Basophiles*

These cells were totally absent from the blood of the first group and within the normal range in the second group.

*Lymphocytes*

The range of these cells varied widely in both groups. In general, however, they were much below the normal average. In the first group they fell as low as 3.2 per cent of the total leucocytes, the highest recorded being 29.2 per cent, with an average of 9.7 per cent. In the second group they ranged between 2.8 and 32.4 per cent of the total leucocytes, with an average of 12.6 per cent.

*Monocytes*

There was no significant change in the average percentage of these cells in either group. The range for the severe was from 0.3 up to 27 per cent, and for the mild from 2.0 up to 26 per cent, with an average of about 10 per cent for the two groups.

*Schilling Index*

Myelocytes as well as juveniles were present in the peripheral blood. The average percentages of myelocytes were 0.25 and of juveniles 2.3, for both groups.

The stabs were highly increased, up to 50 per cent, or even higher, of the total leucocytes, with an average of 19.4 for the severe cases and 11.7 per cent for the mild cases.

The segmented cells were decreased. The range for the severe cases was between 30.7 and 78.6, with an average of 55.7 per cent, and for the mild cases, between 22 to 82 per cent, with an average of 58.3 per cent of the total leucocytes.

### Discussion

In mild cases of pneumonia, as indicated in the above results, the red cell count may fall below three millions, but the average in these cases remains

well within the normal range. The hæmoglobin percentage as well as the colour index are below normal. This denotes that mild anæmia was always present.

Grave cases showed severe anæmia. This was manifested by the reduced red cell count, hæmoglobin percentage, and colour index and increased percentage of reticulocytes.

*Anæmia*

Cabot (1898) noted clinically that there may be a slight anæmia following the crisis in pneumonia and cites Grawitz as stating that the post-critical relaxation of peripheral vessels causes a dilution of the blood with an apparent loss of red cells. Rosenow (1905) cites Boeckman as saying that the number of erythrocytes is proportional to the degree of temperature in pneumonia, and he himself declares that there is scarcely any infection in which the production of anæmia is so rapid as in pneumonia. Chauncey, D., and Leak (1922), on examining the erythrocytes and making blood volume and hæmoglobin determinations in a series of dogs suffering from experimental pneumonia, indicate that in this condition there is actual loss of the circulating erythrocytes without compensatory regeneration.

Kracke and Garver (1937) state that pneumonia produces a mild grade of anæmia with the hæmoglobin diminished to a greater extent than the erythrocytes. The anæmia appears at about the time of convalescence. Normoblasts and reticulocytes are slightly increased.

Piney (1931) notes that changes in the red cells do not occur during the febrile stage, but a few normoblasts may be found. These appear as a result of the great activity of the marrow and denote an effect of compensatory excess of erythropœisis. He adds that anæmia is common in the post-febrile stage but is never severe; it probably results from toxæmia rather than from loss of blood into the exudate, because this is always only slight.

Pepper and Farley (1934), on the other hand, state that anæmia is not a marked feature of the usual case of *lobar* pneumonia, for often only a very trifling reduction in the red cell count and hæmoglobin is found. Occasionally, however, a more rapid reduction appears. Nucleated red cells are rarely seen during the disease but may become numerous following the crisis.

From the above it is obvious that our data agree well with the results of other observers.

*Leucocytosis*

Moreover, our findings denote the presence of leucocytosis in mild cases. This is followed by leucopenia before death in grave cases. It can thus be concluded from these data that leucocytosis is a favourable sign, and where leucopenia exists a grave and fatal termination is to be expected. These results are well cited by many other observers.

Kracke and Garver (1937) state that among the diseases that are characterised by a marked pathological leucocytosis are pneumonia (lobar) and bronchitis, but only if the organisms are of the pyogenic type. The leucocytosis begins early and quickly reaches the maximum and continues high throughout the disease. A steady rise in the leucocyte count during the course of the disease

is to be regarded as favourable. On the other hand, if an initial low leucocyte count shows little or no tendency to rise, the prognosis is grave.

Whitby and Britton (1939) state that the leucocytosis continues during the febrile period until the crisis, but thereafter rapidly returns to a high normal.

Price-Jones, C. (1939), states that probably the most extreme type of leucoid blood picture is met with in cases of acute lobar pneumonia. This picture exhibits an extreme leucocytosis, after showing myelocytes, and is later associated with anæmia.

Gradwohl (1938) states that we can draw conclusions very accurately in many infections from the blood picture; for instance, in pneumonia we find a hyperleucocytosis and a neutrophilia with regenerative shift.

*Leucopenia*

As regards the leucopenia of grave pneumonia, Whitby and Britton (1939) state that the absence of leucocytosis during the febrile phase is sometimes of grave prognostic significance; it cannot be said that the degree of leucocytosis forms a sound basis for estimating the outlook. It is unsound to believe that the higher the leucocytosis the better the prognosis, but it is usual in favourable cases for the count to be of an order from 20,000 to 30,000. He states that leucopenia at the onset is not necessarily a grave prognostic sign; but an initial leucocytosis that declines markedly before the crisis is of serious import.

Meninger (1925) reported a case of extreme leucopenia with a granulocytosis in which death occurred very quickly.

Pepper and Farley (1934) state that the height of leucocytosis has been said to bear a rough relationship to the severity of the disease and the extent of the lung involvement, but the exceptions are too numerous to permit the acceptance of this view. On the other hand, absence of the expected leucocytosis is of grave prognostic significance; a true leucopenia is even graver.

Piney (1931) states that the total number of leucocytes falls suddenly at the time of the crisis to normal, or even lower, while the neutrophiles gradually return to normal percentage proportions.

*Neutrophilia*

It was noticed that it is not only the total number of leucocytes that are increased in mild cases, but there is a relative increase in the percentage of neutrophiles in both groups of cases. During the febrile period they are enormously increased, both absolutely and relatively, often forming more than 80 per cent of the total number of leucocytes.

Whitby and Britton (1939) state that pneumonia is in all favourable cases characterised by a polymorphonuclear leucocytosis of 20,000 cells to 30,000 cells per c.mm., of which some 90 per cent are neutrophiles.

Blount, W. P. (1930 and 1931), states that it is recognised that the majority of acute inflammations are due to cocci—pyogenic—organisms, which have a specific stimulus for the neutrophiles; consequently neutrophilia occurs in the majority of acute infections.

Pepper and Farley (1934) state that there occurs a distinct neutrophile leucocytosis with averages about 25,000. The total count often exceeds 30,000

and may be far above this; with such a count perhaps 90 per cent or more of neutrophiles will be present. A small percentage of myelocytes is very common during the height of any such neutrophilic leucocytosis.

Kilduffe (1931) states that pneumonia is one of the diseases characteristically accompanied by a marked and not infrequently extreme degree of leucocytosis. It is commonly accepted that the degree of leucocytosis is proportionate in a general way to the amount of lung tissue involved, but this is subject to numerous exceptions and is influenced to a marked degree by the reactive ability of the patient.

The following rule, formulated by Sondern (cited by Kilduffe, 1931), will be found to hold good in a large proportion of cases:

1. Slightly increased neutrophilic percentage, regardless of the total count, suggests slight toxic infection; a greatly increased neutrophile count, on the other hand, suggests severe toxic infection.
2. Slight leucocytosis and slight neutrophilic increase suggests slight infection and fair resistance; increased leucocytosis with only slight neutrophilic increase indicates slight infection and good resistance.
3. Slight leucocytosis with a high neutrophilic count suggests severe infection and poor resistance; a high total count under the same circumstances indicates good resistance; absence of leucocytosis suggests no resistance, and a falling total count and rising neutrophile count warrants the inference that the severity of the infection is increasing and the patient's resistance falling.
4. Falling counts suggest decreasing infection and recovery.

The experience of many workers has indicated that there is a relation between the total count and the mortality, the mortality being lower in cases with high total counts.

Piney (1931) states that the leucocytes rise suddenly and considerably at the time of occurrence of the first rigors and continue to rise for some days. The size of the leucocytosis does not always bear a direct relation to the extent of lung tissue involved; it seems to depend on the intensity of the general infection.

The leucocytosis depends entirely upon the increase of the neutrophiles, which show marked or even extreme shift to the left. Many of the neutrophiles also show a positive glycogen reaction. Myelocytes in small numbers are commonly seen. Besides the neutrophilia, it was noticed that the eosinophiles were markedly decreased relatively and absolutely. In some cases they were absent. The lymphocytes were diminished in both groups of cases, while the monocytes showed no significant change.

*Eosinophiles*

Whitby and Britton (1939) state that with regard to the eosinophiles, they disappear from the circulation at the onset of the disease but reappear shortly before or just after the crisis.

Pepper and Farley (1933) state that the eosinophiles disappear; the leucocytes may be reduced in actual number and non-filament nuclear neutrophiles

rise to 40 per cent or more. After the crisis in uncomplicated pneumonia, the total count begins at once to fall with a prompt decrease in the percentage of neutrophiles. Sometimes for a few days there occurs a moderate eosinophilia.

Piney (1931) states that the eosinophiles are usually absent, the leucocytes much reduced and the monocytes decreased, but the last-named cells often rise a little before the occurrence of the crisis. Eosinophiles and lymphocytes rise quite rapidly and post-infective lymphocytosis and eosinophilia are the rule during convalescence.

*Schilling Index.*—It was also noted that all the different indices showed a marked shift to the left in both groups. This is in accordance with the findings of other investigators.

Whitby and Britton (1939) state that prognostic information is said to be given by the Schilling index during the acute phase and by the basophile granule degeneration phenomenon. It is stated that the initial shift to the left which inevitably accompanies the leucocytosis should begin to decrease and bear back towards the right. A continuously increasing shift to the left is unfavourable.

Fairlie Clarke, A. J. (1929), found immediate high leucocytosis, high polymorphonuclear cell percentage and a shift to the left. Leucocytosis remaining high is favourable, but absence of this with increasing shift to the left and appearance of primitive cells is of ill omen.

Kohlman (1931), in a study of 50 cases, using the Schilling index, found that cases in which shift to the left was constantly becoming more marked had a bad prognosis, while those with decreasing shift to the left or beginning shift to the right had a favourable prognosis.

**Summary**

During this study, 26 dogs affected with pneumonia were examined. Ten cases ended fatally and the remaining 16 were destroyed after obtaining full hæmatological records.

All cases showed typical symptoms and lesions of pneumonia affecting one or both lungs. Abscesses were met with in some cases.

The blood picture for mild cases showed a slight anæmia, marked leucocytosis with neutrophilia, both relative and absolute, and marked eosinopenia and lymphopenia, with no change in the monocytes.

Severe cases, on the other hand, showed severe anæmia with marked decrease in hæmoglobin, a low colour index and a rise in the reticulocytes. The anæmia was accompanied by marked leucopenia with relative neutrophilia and marked decrease in the eosinophiles and lymphocytes. The monocytes were not significantly affected.

The Schilling index showed a marked shift to the left.

One may thus conclude that leucocytosis is a favourable prognostic feature, while leucopenia is to be considered a grave sign, pointing to a fatal termination.

**(b) Distemper**

Eighteen male and female dogs, varying in age from six months to one-and-a-half years, were examined in this study. The symptoms and lesions

seen on post-mortem examination in all cases in varying degree were characteristic of a true distemper infection.

## Observations and Results

*Red Blood Corpuscles*

The average red cell count was 4,410,000 per c.mm., but varied from 1,340,000 to 6,570,000 per c.mm.

*Hæmoglobin Percentage*

The average was 46 per cent, the calculated limits being 15 to 80 per cent.

*Colour Index*

This ranged from 0.38 to 0.75 with an average of 0.52.

*Reticulocytes*

The count was very often high, ranging from 0.0 to 15 per cent, with an average of 1.9 per cent.

*Total Leucocyte Count*

The number of the leucocytes varied from 3,200 to 20,000, with an average of 12,000.

### Differential Leucocyte Count

*Neutrophiles*

Relative and absolute neutrophilia was present in advanced cases, the neutrophiles ranging from 35 to 93.3 per cent of the total leucocytes.

*Eosinophiles*

These were within normal range or slightly increased to 21 per cent, with an average of 6.3 per cent in all the cases. In fatal cases the eosinophiles decreased or were totally absent.

*Basophiles*

These were absent from the peripheral blood of all cases.

*Lymphocytes*

Lymphopenia was recorded, the average percentage of the lymphocytes falling to 10.4 per cent and ranging from 4.8 to 19 per cent.

*Monocytes*

Monocytosis was marked in all cases, especially those terminating fatally, the monocytes being present to the maximum extent of 32 per cent of the total count with an average of 12.8 per cent.

In these cases the index revealed the presence of young and band forms of neutrophiles to a great extent. Myelocytes were present up to 1 per cent, with an average of 0.2 per cent; the juveniles reached, in one case, 5 per cent, the average for all cases being 1.5 per cent of the total leucocytes. The stab forms were much increased in many cases; 52.3 per cent was recorded in one case. The average stab percentage reached 12.2 per cent. The mature cells amounted to 56.6 per cent. This fact that the stab cells were increasing and the mature cells decreasing conjointly, with the clinical manifestations, pointed to the severity of the attack in some cases and a fatal termination was expected.

XUM

## Discussion

The hæmatologic picture which accompanies distemper varies widely, according to the severity of the condition and to the different complications of the disease. A mild anæmia, becoming severe, as a rule develops. The condition deteriorates but the picture always remains that of a secondary type of anæmia with a low colour index. The red cell count is markedly decreased; counts as low as 1,340,000 cells per c.mm. were encountered. The hæmoglobin percentage is correspondingly decreased and reticulocytes increased in the peripheral blood. Films showed anisocytosis, poikilocytosis and the presence of nucleated red cells. The leucocytes reached the high level of 20,000 per c.mm.

The above described anæmia may be due to several factors, the acute infection and gastro-intestinal dysfunction being the most important of all.

Kracke (1937) states that varying degrees of anæmia are seen in practically all types of acute infections, particularly if the infection is widespread or systemic in nature. This type of anæmia is apparently brought about by various modes of action. The infecting organism, particularly if in considerable numbers in the blood stream, may exert a destructive effect on red cells with which it comes in contact. Furthermore, its toxins may be capable of a similar effect. As to other factors, he reports that varying degrees of hypochromic anæmia may occur as a result of disorders of the stomach or intestines whether or not accompanied by blood loss. Furthermore, it is not necessary that organic pathology should exist in order to produce anæmia because functional disorders such as hypermotility of the intestinal tract may, by itself, prevent the absorption of an adequate amount of iron and other agents necessary for building the hæmoglobin molecule. Varying degrees of hypochromic anæmia have been reported in such diverse conditions as diarrhœa and dysentery of all kinds.

There may be another possible predisposing factor for this anæmia. De Camp, C. E. (1936), suggests that the low hæmoglobin and the red cell content of puppies under a year old undoubtedly is due in large measure to the milk diet on which animals of this age subsist to a large extent. The use of iron in these animals will correct these abnormalities and improve the general condition of these cases.

Our results agree well with those of Sabrazès and Muratet (1906), who examined the blood of four cases of dog distemper at Bordeaux, where the nervous form of the disease is frequently observed and is very severe, the mortality exceeding 60 per cent. The counts of the red blood corpuscles and leucocytes and the amount of hæmoglobin were not reported. "In one case there were signs of anæmia with normoblastic reaction. In another case there was slight anisocytosis and polychromatophilia. The changes in the leucocytes were more pronounced. Polynucleosis with lessened number of eosinophiles was the rule. The absolute number of leucocytes was increased in the three cases."

## Summary

Eighteen young dogs varying in age from 6 to 18 months were found affected with canine distemper which was confirmed clinically and on post-

mortem examination. The blood showed a secondary anæmia, with low red cell count, decreased hæmoglobin amount and colour index. Reticulocytes and nucleated red cells accompanied by anisocytosis and poikilocytosis were detected in the peripheral blood.

The leucocytes were increased in number, with neutrophilia and monocytosis, which were accompanied by lymphopenia. The eosinophiles were within the normal range or slightly decreased following complications.

The young and stab forms of neutrophiles were increased, while a decrease in the segments was observed.

The Schilling index revealed a marked shift to the left for the neutrophiles.

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# *REVIEWS*

THE LIVING SOIL, by Lady Eve Balfour. Pp. 270. Price 15s. London: Faber & Faber, Ltd.

A NEW ninth edition of Lady Eve Balfour's remarkable book, "The Living Soil," is most welcome for she is engaged on producing 'evidence of the importance to human health of soil vitality' (to quote her sub-title), and this evidence is equally important to animal health. If her case is proved, and it has advanced far along the line to finality, she will have accomplished something which will affect the whole future of mankind. She sums up her intentions in writing her book in these words:—

> "One of my objects in undertaking this book has been to bring together, within the covers of a single volume, a summary of certain recent scientific research on nutrition and on soil fertility, including results that have been obtained in many different parts of the world by practical application of the principles involved."

The reader will therefore find widely scattered information gathered together and presented in an easily readable form. Whether he be a layman or a scientist he will find he cannot but go on to the end once he starts out under Lady Eve Balfour's guidance. Step by step he is taken through the medical evidence, humus, direct evidence as shown in soil ecology, evidence from experiments in the feeding of plants, evidence from animal feeding experiments, effects of diet on human health, the problem of producing indisputable proof, and so on, to a chapter headed "Facing the Implications." Everything that grows is the product of what it is nourished with. Poor land spells poor crops: poor crops produce poor animals: disease is eliminated or vastly reduced under good nutrition: conversely, it is induced by lack of essential nutritional elements: all these things are truisms and yet it is essential to prove them and especially to establish what constitutes good nutrition. It would be difficult to find a better, more clearly presented or more scientific a presentation of the new outlook regarding agriculture and its bearing on human and animal health than this book offers. As a private individual, as a thinking citizen, and as a scientist, every veterinary surgeon and every veterinary student should read this book.

EXPERIMENTAL SURGERY, by J. Markowitz, M.D.E., M.B., Ph.D. Pp. xiv — 546, with 335 figures. Second edition. Price 40s. net. London: Baillière, Tindall & Cox.

A GREAT surgeon, a man of international renown, once said: "I have learnt nothing of value to my art as a result of experiments on dogs." To-day such a statement would be classed as ridiculous. Modern surgery owes much to those who have conducted their original investigations on dogs. For this we must give due credit to the experimental physiologists, for they have opened the gate by which the surgeon may enter the field. Operations are now performed on man, and with successful results, which but a few years ago would have been beyond belief. One can cite operations on the nervous system, the circulatory system, the endocrine organs, the liver, the lungs, as typical examples of the above statement.

No doubt the old surgeon did not need to have recourse to dogs for in his time he could conduct his surgical experiments upon the patients who thronged the wards and out-patient department of any general hospital. Nowadays, a different conception of life exists, and when other means are at hand men and women are not used as the subjects of wholesale biological experiments. There is no need to apply the risks of speculative surgery to them until the fundamental principals have been worked out and justified on lesser vertibrates.

The anatomists and the physiologists were the pioneers in the field and many acquired a skill and precision in their work which provoked the envy of the surgeon. The latter has not been slow to react and to recognise that apart from any increase in the fund of academic knowledge here was a method of enhancing their skill and craft with great faciltiy and with benefit to their subsequent human patients.

A surgeon is a craftsman, and his results depend to a great extent upon his skill and technique.

Markowitz, a physiologist, was one of the first to put his experimental surgical experiences on record, and his work is of classic standard. The experienced surgeon as well as the tyro can benefit by the perusal of his book. This is particularly so as regards technique. The book is crammed with excellent illustrations which can be understood in many cases without reference to the text.

This is a book which one can recommend to any surgeon who desires to enhance his efficiency. The enthusiast will find it thrilling and stimulating.

NEOPLASMS OF THE DOG, by R. M. Mulligan (1949). Pp. xi—135 and 59 plates. Price 31s. 6d. net. London: Baillière, Tindall & Cox.

ESSENTIALLY, this book is a careful and detailed account of the macroscopic and microscopic features of the different types of neoplasm in a consecutive series of one thousand specimens of tumours from dogs out of a total of over 1,500 that the author has studied. In an introductory chapter, after a brief definition of terms such as neoplasia and malignancy, tables are given showing the ages of the 809 dogs from which the tumours came, the breeds of 669 of these animals, and then a classification, according to type, of the 1,000 tumours. Features of age, sex, and breed incidence are referred to again later in connection with the different types of tumour. In the succeeding chapters, the author gives an account of the following tumours: mammary neoplasms; epithelial neoplasms of the skin and related structures; neoplasms of mesenchymal origin and of nerve sheath cells; melanoma; mast cell sarcoma, lymphosarcoma, histiocytoma; neoplasms of endocrine glands (especially those of testis, ovary and thyroid gland). In a concluding chapter are described those tumour types of which only a very few examples were encountered by the author. A technical supplement gives a method of packing and forwarding tumour specimens, and describes some staining methods the author has used. There are 59 plates of black and white illustrations, showing macroscopic and microscopic features of most of the tumours dealt with; these illustrations are a striking and very useful feature of the book. In the various chapters, except the last, one notices the almost complete lack of references to other workers' accounts of the neoplasm under consideration, but there is a fairly extensive bibliography at the end of the book. The writing is often very condensed, and it is necessary for the reader to supply mentally the words "in this series of tumours" in the case of some of the statements made.

In the preface, Prof. Mulligan states that "a search of the literature has demonstrated the need for a book on the neoplastic diseases of the dog for the use of the student and of the practitioner as well as of the teacher in veterinary pathology." Of the student, however, a good prior knowledge of the pathology of tumours in general would be required for him to appreciate the book fully, and he might find the histological details rather heavy going. Again, to the

practitioner, the references that are made to clinical features and to methods of treatment may sometimes seem too brief, and he would probably like to see included some representative and fully detailed accounts of individual pre- and post-operative clinical histories, together with more detailed methods of operation.

In future editions, which will certainly be called for, the author, while rightly basing his work on his own studies of the still larger number of tumours he will have examined, may feel able to give himself more elbow room, and write a more widely-ranging account, with more extensive and critical reference to other work, and perhaps longer clinical accounts. This is, of course, not to say that the present book will not well repay reading by all interested in the tumours of the dog. As an account of the morbid anatomy and histopathology of a series of neoplasms of the dog, the book will be of great interest to the pathologist, medical as well as veterinary.

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## *NOTICES*

BURROUGHS WELLCOME AND CO. announce the introduction of "Wellcome" brand Leptospira Canicola Vaccine for the prevention of Canicola fever in the dog. The new product is issued in containers of 10 c.c. They also announce that they have available an important new agent for the treatment of roundworm infestation in puppies and cats. It is issued as "Banocide" brand Diethyl Carbamazine (Veterinary) in tubes of 10, and has been shown to be particularly efficient and safe when used in young puppies. Further particulars will be supplied on request.

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THE DISTILLERS COMPANY (BIOCHEMICALS), LTD., of Speke, Liverpool, announce that, as from February 27, 1950, they will reduce Crystalline Penicillin G to the price of Amorphous (Coloured) Penicillin:—

100,000 i.u., single vial, 1s. 3d.; box of 5 vials, 6s. 3d. 200,000 i.u., single vial, 1s. 10½d.; box of 5 vials, 9s. 4½d. 500,000 i.u., single vial, 3s. 6d.; box of 5 vials, 17s. 6d. 1,000,000 i.u., single vial, 6s. 6d.; box of 5 vials, 32s. 6d. 5 mega units, single container, 31s. 3d.; 10 mega units, single container, 60s. All prices subject to the usual discounts.

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